

Supplementary Information

Nine undescribed sesquiterpenoids from the aerial parts of *Daphne penicillata*: cyclooxygenase-2 inhibition, molecular docking and molecular dynamics studies

Peng Zhao,^a Ben-Song Xin,^a Feng-Ming He,^b Li Ye,^a Zhen-Tao Ma,^a Jin-Le Hao,^c Rui Shi,^d Xia-Hong He,^d
Guo-Dong Yao,^a Bin Lin,^c Xiao-Xiao Huang,^a Shao-Jiang Song,^{a,*}

^a *Key Laboratory of Computational Chemistry-Based Natural Antitumor Drug Research & Development, Liaoning Province; Engineering Research Center of Natural Medicine Active Molecule Research & Development, Liaoning Province; Key Laboratory of Natural Bioactive Compounds Discovery & Modification, Shenyang; School of Traditional Chinese Materia Medica, Shenyang Pharmaceutical University, Shenyang, Liaoning 110016, China*

^b *School of Pharmaceutical Sciences, Xiamen University, Xiamen, 361102, China*

^c *Wuya College of Innovation, Shenyang Pharmaceutical University, Shenyang 110016, China*

^d *Key Laboratory for Forest Resources Conservation and Utilization in the Southwest Mountains of China, Ministry of Education, International Ecological Forestry Research Center of Kunming, Horticulture and Landscape Architecture, Southwest Forestry University, Yunnan Kunming, 650224, China*

*Corresponding authors.

E-mail: songsj99@163.com (S. J. Song)

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1. Supplementary 1

1.1 Plant material.

The herbs of *Daphne penicillata* were collected from Wenchuan County, Aba Prefecture, Sichuan Province, China, in July 2019 (N31°29'51.52", E103°38'14.35") and authenticated by Prof. Jincui Lu (School of Traditional Chinese Materia Medica, Shenyang Pharmaceutical University). The voucher sample (No. MJ20190701) was deposited in the herbarium of Shenyang Pharmaceutical University.

1.2 Extraction and isolation

The dried herbs of *Daphne penicillata* (48.0 kg) were extracted under reflux conditions using 75% aqueous EtOH (50 L × 3 times × 4 hours). The percolates were combined and evaporated under reduced pressure to afford a crude extract. The extract was suspended in water and successively partitioned with EtOAc and *n*-BuOH. The EtOAc extract (600 g) and *n*-BuOH (1200 g) were subjected to silica gel column using CH₂Cl₂-MeOH (100:1-1:1, *v/v*) as gradient eluents to afford four fractions (Fr.A-D). Fr.C (150 g) was separated by polyamide chromatography eluted with 30% and 80% EtOH to obtain two fractions (Fr.C1 to Fr.C2), then Fr.C1 (75 g) was subjected to column chromatography on HP20 resin with H₂O, 30%, 60% and 90% EtOH to give two fractions (Fr.C1-1 and Fr.C1-3). Fr.C2-1 (28 g) were separated by ODS column chromatography, eluted with a gradient of MeOH-H₂O from 20:80 to 60:40, respectively, and then redistributed in five fractions (Fr.1-6) based on HPLC analysis. Among them, Fr.1 (15 g) was subjected to silica gel CC eluting with petroleum ether/EtOAc (*v/v*, 100:1 to 5:1) to yield five fractions (Fr.1.1–Fr.1.8). Fr.1.5 (2.4 g) and Fr.1.6 (3.5 g) were separated by preparative HPLC using MeOH/H₂O (48:52) to obtain the following fractions (Fr.1.2.1–Fr.1.5.8 and Fr.1.6.1–Fr.1.6.6). Fr.1.2.2 (98.2 mg) and Fr.1.2.3 (65.6 mg) were separated by semipreparative HPLC with CH₃CN-H₂O (20:80, 2.5 mL/min) to afford **1** (1.5 mg, *t_R* = 24.7 min), **2** (3.5 mg, *t_R* = 20.2 min), **3** (4.5 mg, *t_R* = 24.3 min), **4** (8.3 mg, *t_R* = 19.3 min), and **5** (7.7 mg, *t_R* = 34.2 min) was obtained from Fr.1.6.2 (51.8 mg) and Fr.1.6.3 (62.3 mg) by semipreparative HPLC (CH₃CN-H₂O, 25:75, 2.5 mL/min). Fr.1.8 (1.2 g) was separated by RP-C18

preparative HPLC using MeOH/H₂O (47:53) to obtain the following fractions (Fr.1.8.1–Fr.1.8.5). Among them, Fr.1.8.4 (50.2 mg) and Fr 1.8.5 (24.5 mg) were separated by semipreparative HPLC with CH₃CN-H₂O (28:77, 2.5 mL/min) to afford **7** (7.0 mg, *t_R* = 11.2 min), **8** (6.2 mg, *t_R* = 14.7 min), **9** (11.5 mg, *t_R* = 16.6 min), **10** (12.0 mg, *t_R* = 25.4 min) and **11** (33.2 mg, *t_R* = 39.2 min).

1.3 NMR calculations

The conformational analysis of isolated compounds was performed on the CONFLEX software by using MMFF94s molecular force field [1]. All the conformers whose Boltzmann distribution is more than 1% were chosen, and then they were initially optimized at B3LYP/6-31G(d) level in chloroform using the polarizable continuum model (PCM) solvent model by Gaussian 09 program package [2]. The gauge independent atomic orbital (GIAO) shielding constants of these conformers were calculated at the mPW1PW91/6-311+G(d,p) level after geometry optimization. Boltzmann-weighted averages of the chemical shifts were calculated to scale them against the experimental values. The ¹H NMR and ¹³C NMR chemical shift of tetramethylsilane were calculated at the same level and used as reference. The shielding constants obtained were converted into chemical shifts by referencing to TMS at 0 ppm ($\delta_{\text{cal}} = \sigma_{\text{TMS}} - \sigma_{\text{cal}}$). The MAE_{ΔΔδ} values, DP4+ and CP3 probability were calculated for evaluation of the deviations between the experimental and calculated results.

1.4 ECD calculations

Conformational analysis of all the possible conformers of isolated compounds were performed by using the MMFF94 force field in CONFLEX software. All the conformers obtained were screened based on the energy of optimized structures at the B3LYP/6-31G(d) level in an energy window of 3 kcal/mol in the Gaussian 09 software. Then, the ECD data of all the selected conformers were calculated with the time-dependent density functional theory (TDDFT) method at the B3LYP/6-311+G(2d, p) levels with the CPCM model in methanol solution. Finally, the Boltzmann-averaged ECD curve was generated using SpecDis 1.51.

1.5 NO releasing inhibition assay

The inhibition of NO production was assessed by measuring the level of nitrite (NO₂⁻) using Griess

reaction (Li et al., 2015; Maeda et al., 2013). The RAW264.7 cells were seeded in 96-well plate (5×10^5 cells/well) overnight and then incubated with different concentrations of test compounds (3, 10, 30, 100 μM) for 40 min. After pre-incubation with LPS (10 ng/mL) at 37 °C for 24 h, the culture supernatant was transferred to the enzyme-labelled plates and mixed with an equal volume of Griess reagent. 10 min later, the absorbance was measured at 540 nm on a microplate reader. Each experiment was repeated at least three times. Fresh culture medium was used to be control group in all experiments.

1.6 COX-2 inhibitory activity

In vitro COX-2 activity assay was performed by using cyclooxygenase 2 Inhibitor Screening Kit (Beyotime, Cat No. S0168, China). COX-2 assay buffer (75 μL), COX-2 cofactor working solution (5 μL) and COX-2 working solution (5 μL) were pre-incubated with serially diluted compounds (5 μL) for 10 min at 37°C, then the COX-2 substrate (5 μL) and COX-2 probe (5 μL) were added for another 15 min incubation at 37°C. The optical density (OD) values were thereafter measured with the excitation wavelength at 560 nm and the emission wavelength at 590 nm respectively by Microplate Reader (BioTek, Synergy 2). The data were analyzed using GraphPad Prism8 (GraphPad Software Inc.). All the tests were performed in triplicates.

1.7 Molecular docking

In this study, the crystal structure of COX-2 in complex with celecoxib (PDB ID: 3LN1) was obtained from the PDB database (<https://www.rcsb.org/>). The structure was prepared using the Protein Preparation Wizard [3] module of Schrödinger Suite 2021-2 with default settings. This included removing water and solvent molecules, adding hydrogen atoms, completing missing side chains and loops, optimizing hydrogen bond networks, and energy minimization. To prepare the small molecule structures for docking, the LigPrep (LigPrep, Schrödinger, LLC, New York, NY, 2021) module with default settings was used to convert the 2D structures to 3D structures. The Induced Fit Docking (Induced Fit Docking, Schrödinger, LLC, New York, NY, 2021) tool was then applied to dock the target compounds to the protein by the standard protocol. The center of the docking box was placed at the position of the native ligand celecoxib, and other docking parameters were set to default values. To evaluate the reliability of the docking method, prior to docking the active compounds, the native ligand celecoxib was redocked into the active site of COX-2. The top-ranked docking pose had an RMSD of 0.8039 Å compared to the native ligand pose, indicating the accuracy of the docking

method. Next, the five active compounds in the study were docked individually into the active pocket where celecoxib binds to explore potential modes of interaction between the small molecules and the protein. Finally, the simulation results were analyzed and visualized using the Maestro (Maestro, Schrödinger, LLC, New York, NY, 2021) of Schrödinger and Pymol (The PyMOL Molecular Graphics System, Version 2.3 Schrödinger, LLC).

1.8 Molecular dynamics simulation

Binding Pose Metadynamics is an enhanced sampling method that allows for the evaluation of the binding stability of small molecule ligands to proteins in aqueous solutions within a short simulation time. In this study, we utilized Schrödinger's Binding Pose Metadynamics tool to investigate the stability of compounds **3** and **4** while binding to the COX-2 active site in an aqueous solution. The simulation parameters were set to their default values, with the exception of the number of trials per pose, which was set to 10.

The OLPS4 force field [4] in Desmond [5,6] software was used to simulate the compound **3**/COX-2 and compound **4**/COX-2 complexes. These complexes were dissolved in a cubic simulation box with periodic boundary conditions. The box boundaries were set to be 10 Å away from the complexes, and K⁺ and Cl⁻ ions were placed in the water phase to achieve an experimental salt concentration of 0.15 M KCl and neutralize the system. To ensure the accuracy of the simulation, the entire system underwent equilibration using a default protocol consisting of a series of restrained minimizations and MD simulations before the final production [7]. A 100 ns unconstrained production run was then performed using the NPT ensemble (constant number of atoms N , pressure P , and temperature T), resulting in 1000 frames. The particle mesh Ewald (PME) method [8,9] was used to calculate long-range electrostatic interactions, with van der Waals forces set to a cutoff of 9 Å. The Nose-Hoover chain thermostat and Martyna-Tobias-Klein barostat were used to maintain the temperature and pressure at 310K and 1.01325 bar, respectively. To analyze the trajectories, the Simulation Interaction Diagram tool was used to perform RMSD analysis of protein backbone atoms and ligand heavy atoms, as well as to analyze protein-ligand interactions. Principal component analysis was performed on the trajectories using Schrödinger's trj_essential_dynamics script, and the free energy landscape was plotted using the gmx sham command in GROMACS (V. 2022.1) [10,11] based on PC1 and PC2 of protein C α motion, with the temperature set to 310 K used in the MD simulation. Additionally, the Schrödinger-provided thermal_mmgsa.py script was used to calculate the binding free energy of compounds 19 and 21 to COX-2.

1.9 Cell culture

Hep3B, HepG2 cell lines were obtained from the America Type Culture Collection (USA). The cells were cultured in Dulbecco's modified eagle medium (DMEM) (Hyclone, USA) supplemented with 10% fetal bovine serum (Biological Industries, Israel) in 37 °C, 5% CO₂ incubator.

1.10 Cytotoxic activities

Cytotoxic activities of isolated sesquiterpenes against Hep3B and HepG2 cells were evaluated using an MTT assay. The cells were treated with different concentrations of each compound or DMSO. After 48 h, 20 μ L MTT (5 mg/mL) was added to each well and incubated for 4 h at 37 °C. The medium was removed and DMSO (150 μ L/well) was added to each well, and cell viability was measured using a microplate reader (Thermo, Carlsbad, CA, USA) at 490 nm. All tests needed three separate experiments. The IC₅₀ values were calculated using GraphPad Prism 6 software.

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2. Supplementary 2

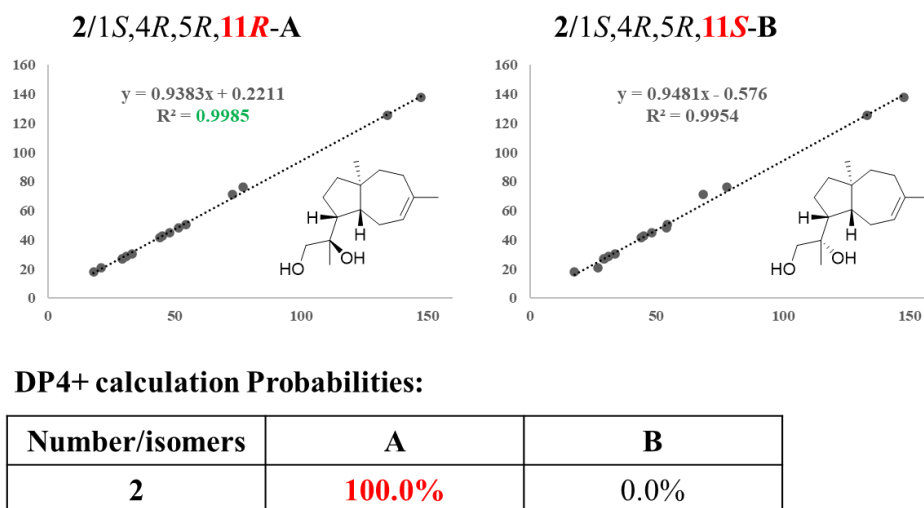


Figure S1. The relative configurations determination of **2** by R^2 and DP4+ analysis.

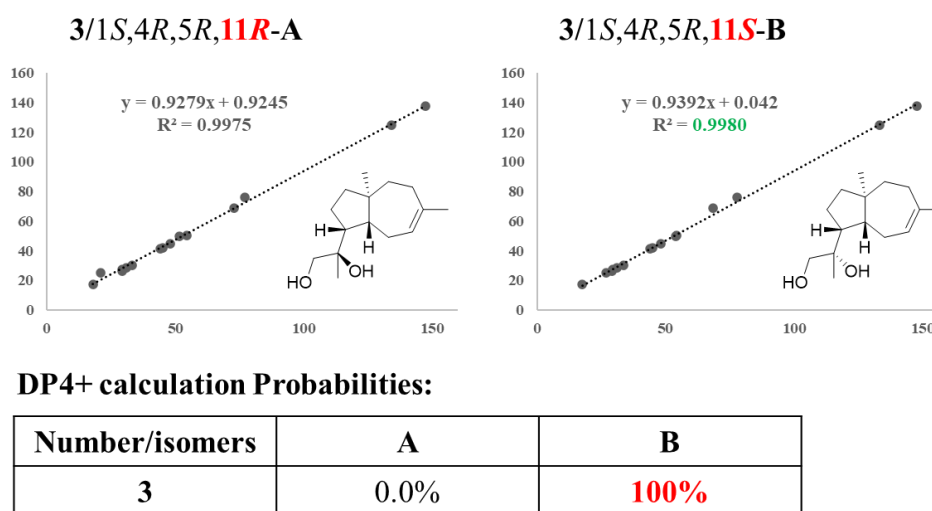


Figure S2. The relative configurations determination of **3** by R^2 and DP4+ analysis.

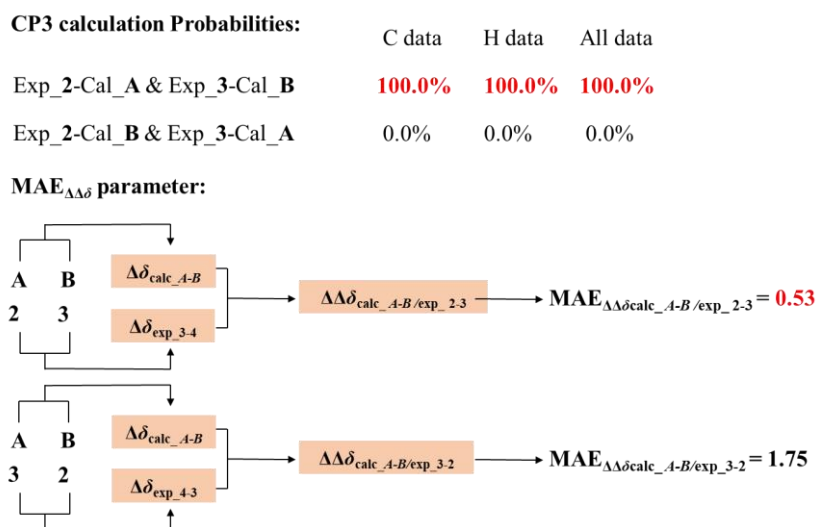


Figure S3. The relative configurations determination of **2** and **3** by MAE $_{\Delta\Delta\delta}$ and CP3 analysis.

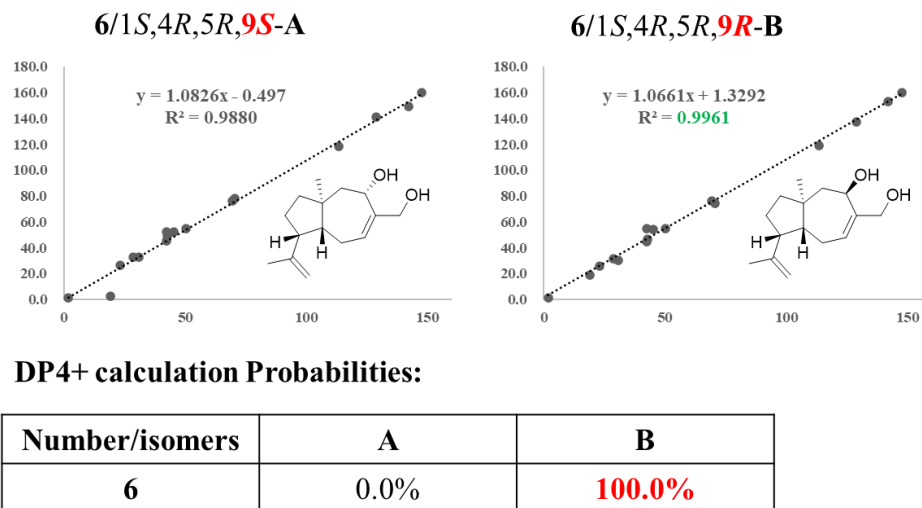
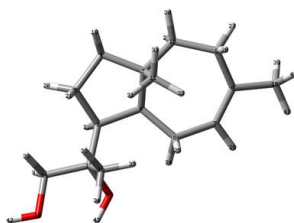


Figure S4. The relative configurations determination of **6** by R^2 and DP4+ analysis.

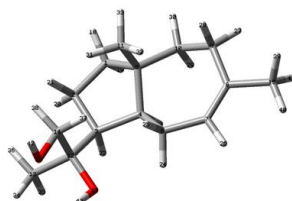
2/3-A



Weighting factors:

1	16.59016
2	16.84617
3	18.55616
4	19.31377
5	18.94808
6	3.86710
7	1.65443
8	0.16189
9	0.18531
10	0.31336
11	0.03308
12	1.65455
13	1.37656
14	0.10705
15	0.02496
16	0.03641
17	0.01433
18	0.02811
19	0.01219
20	0.19273
21	0.02398
22	0.02494
23	0.03464

2/3-B



Weighting factors:

1	23.08932
2	20.31467
3	38.93488
4	13.10740
5	0.36030
6	3.26870
7	0.41868
8	0.02259
9	0.21414
10	0.26933

Figure S5. The weighting factors of structures **2/3-A** and **2/3-B** for NMR calculation.

	2	3		A	B
1	42.4	42.1	1	45.2	44.9
2	26.8	26.2	2	29.3	29.3
3	48.1	49.8	3	51.6	54.1
4	50.7	50.4	4	54.5	54.2
5	44.6	44.6	5	48.0	48.1
6	26.9	27.6	6	29.4	29.4
7	125.4	124.7	7	134.1	133.5
8	137.5	137.6	8	147.5	147.9
9	30.4	30.4	9	33.3	33.5
10	41.3	41.3	10	44.2	44.1
11	17.9	17.5	11	18.0	17.7
12	76.2	76	12	77.2	78.0
13	21	25	13	21.0	26.8
14	71	68.8	14	72.8	68.5
15	28.5	28.5	15	30.9	31.0
18	1.45	1.45	18	1.44	1.35
19	1.32	1.32	19	1.33	1.27
20	1.62	1.68	20	1.43	1.53
21	1.31	1.54	21	1.25	1.34
22	2.45	2.44	22	2.35	2.34
23	1.91	1.85	23	1.82	1.71
24	2.77	2.55	24	3.03	3.16
25	2.15	2.07	25	2.22	2.15
26	5.51	5.46	26	5.98	5.96
27	1.89	1.89	27	1.86	1.80
28	2.25	2.21	28	2.36	2.34
29	1.25	1.24	29	1.19	1.12
30	1.67	1.66	30	1.59	1.58
31	0.88	0.84	31	0.89	0.78
32	0.88	0.84	32	0.89	0.78
33	0.88	0.84	33	0.89	0.78
34	1.19	1.26	34	1.09	1.12
35	1.19	1.26	35	1.09	1.12
36	1.19	1.26	36	1.09	1.12
37	3.4	3.61	37	3.29	3.62
38	3.28	3.49	38	3.16	3.47
39	1.7	1.69	39	1.77	1.78
40	1.7	1.69	40	1.77	1.78
41	1.7	1.69	41	1.77	1.78

Figure S6. Calculated ^{13}C NMR chemical shifts of two candidate structures (**A-B-2/3**)

1	2					
2	3	$\Delta\delta_{\text{exp1-2}}$	$\Delta\delta_{\text{exp2-1}}$	A	B	$\Delta\delta_{\text{calcA-B}}$
42.4	42.1	0.3	-0.3	45.23389	44.87251	0.36138
26.8	26.2	0.6	-0.6	29.26103	29.29642	-0.03539
48.1	49.8	-1.7	1.7	51.56225	54.05787	-2.49562
50.7	50.4	0.3	-0.3	54.47373	54.22769	0.24604
44.6	44.6	0	0	48.01401	48.05152	-0.03751
26.9	27.6	-0.7	0.7	29.44981	29.40908	0.04073
125.4	124.7	0.7	-0.7	134.07495	133.49199	0.58296
137.5	137.6	-0.1	0.1	147.54969	147.91203	-0.36234
30.4	30.4	0	0	33.31283	33.53201	-0.21918
41.3	41.3	0	0	44.18437	44.13517	0.0492
17.9	17.5	0.4	-0.4	17.98244	17.68752	0.29492
76.2	76	0.2	-0.2	77.23448	78.00465	-0.77017
21	25	-4	4	21.02985	26.84963	-5.81978
71	68.8	2.2	-2.2	72.75429	68.45963	4.29466
28.5	28.5	0	0	30.88507	31.00766	-0.12259

$\Delta\Delta\delta_{\text{calcA-B/exp1-2}}$		$\Delta\Delta\delta_{\text{calcA-B/exp2-1}}$		
0.06138	0.06138	0.66138	0.66138	
-0.63539	0.63539	0.56461	0.56461	$\text{MAE}\Delta\Delta\delta_{\text{calcA-B/exp1-2}}$
-0.79562	0.79562	-4.19562	4.19562	0.538975333
-0.05396	0.05396	0.54604	0.54604	
-0.03751	0.03751	-0.03751	0.03751	
0.74073	0.74073	-0.65927	0.65927	
-0.11704	0.11704	1.28296	1.28296	$\text{MAE}\Delta\Delta\delta_{\text{calcA-B/exp2-1}}$
-0.26234	0.26234	-0.46234	0.46234	1.758682
-0.21918	0.21918	-0.21918	0.21918	
0.0492	0.0492	0.0492	0.0492	
-0.10508	0.10508	0.69492	0.69492	
-0.97017	0.97017	-0.57017	0.57017	
-1.81978	1.81978	-9.81978	9.81978	
2.09466	2.09466	6.49466	6.49466	
-0.12259	0.12259	-0.12259	0.12259	

Figure S7. $\text{MAE}_{\Delta\Delta\delta}$ parameter between the experimental and calculated ^{13}C NMR chemical shifts between 2/3 and A/B

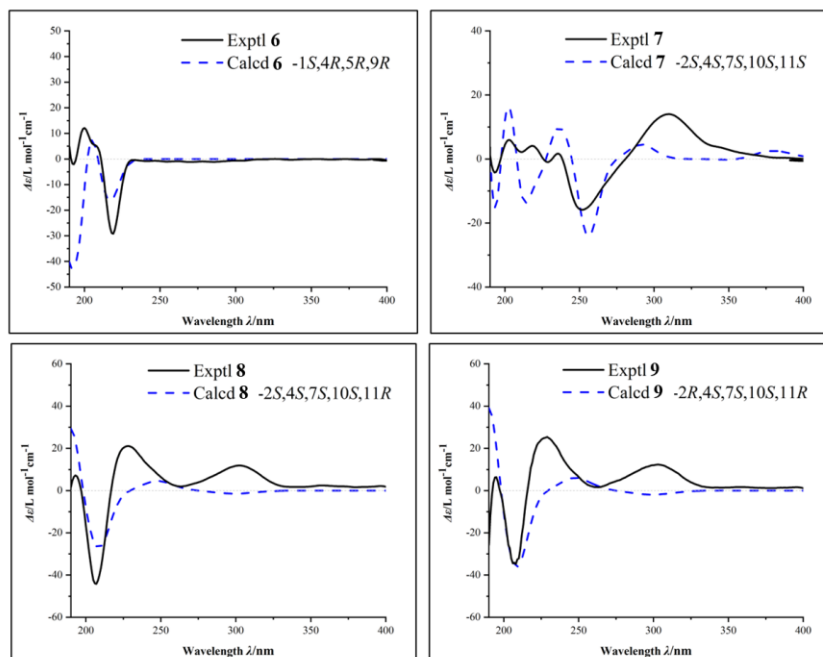


Figure S8 The calculated ECD and experimental spectra of 6-9.

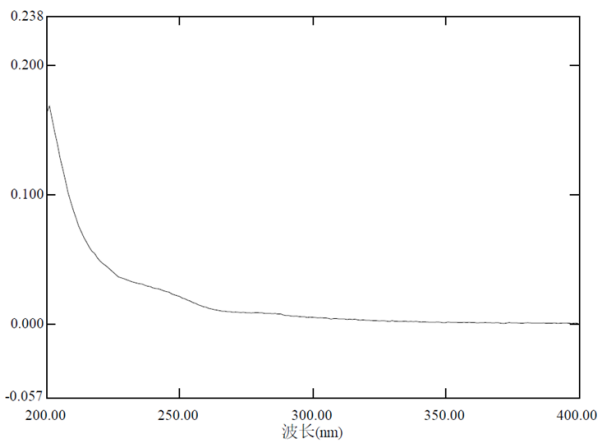
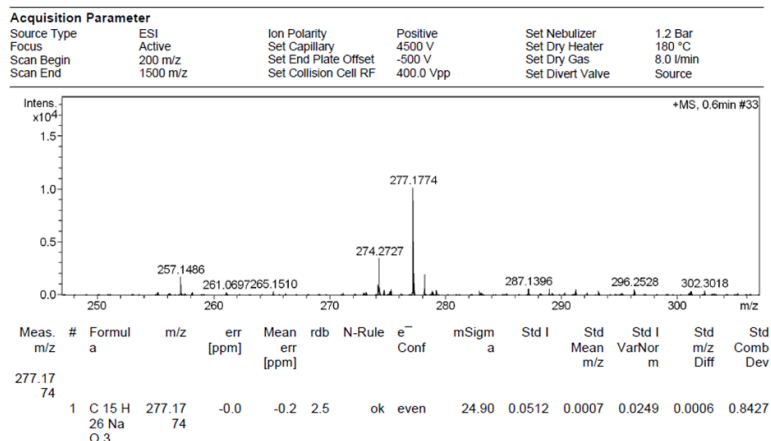


Figure S9 HRESIMS and UV spectra of compound 1

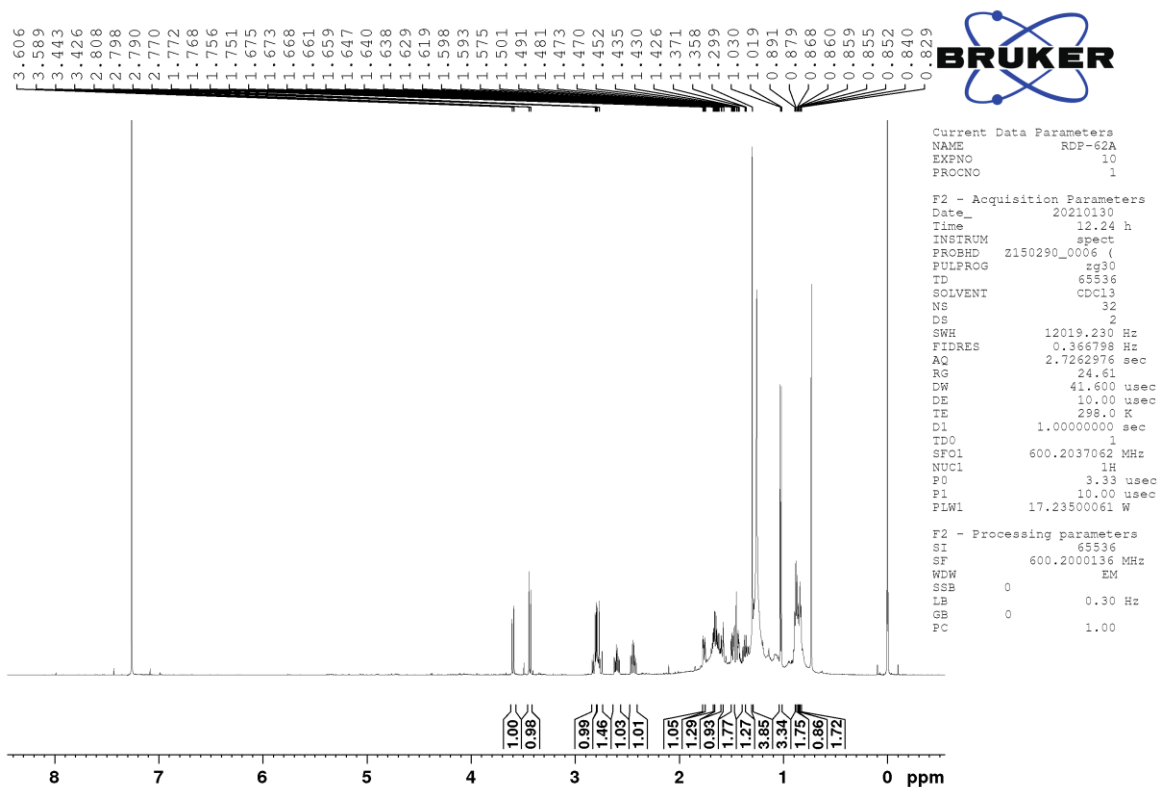


Figure S10 ¹H NMR spectrum (600 MHz, CDCl₃) of compound 1

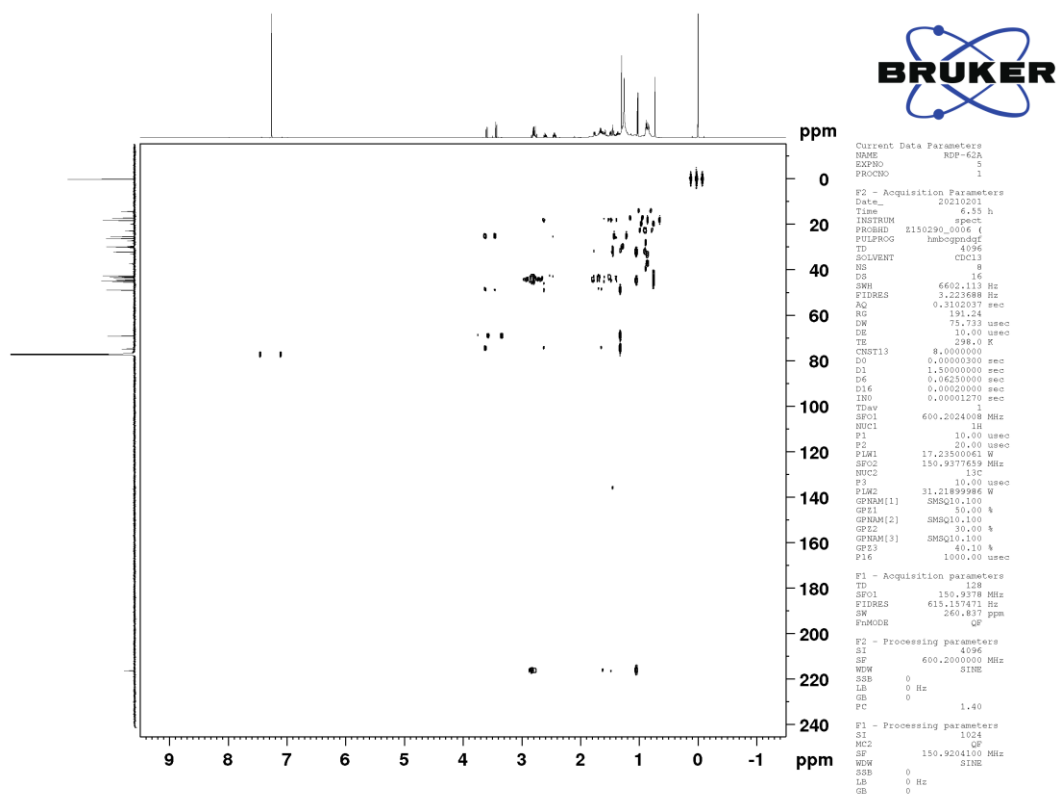


Figure S13 HMBC spectrum (600 MHz, CDCl₃) of compound 1

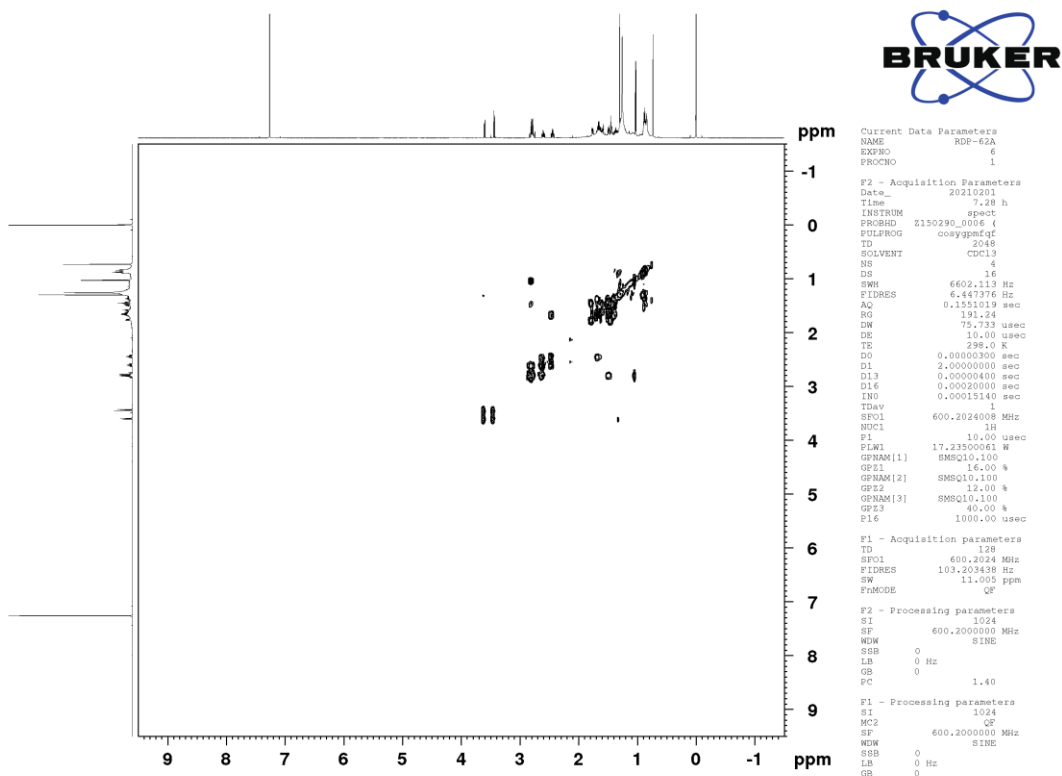


Figure S14 ¹H-¹H COSY spectrum (600 MHz, CDCl₃) of compound 1

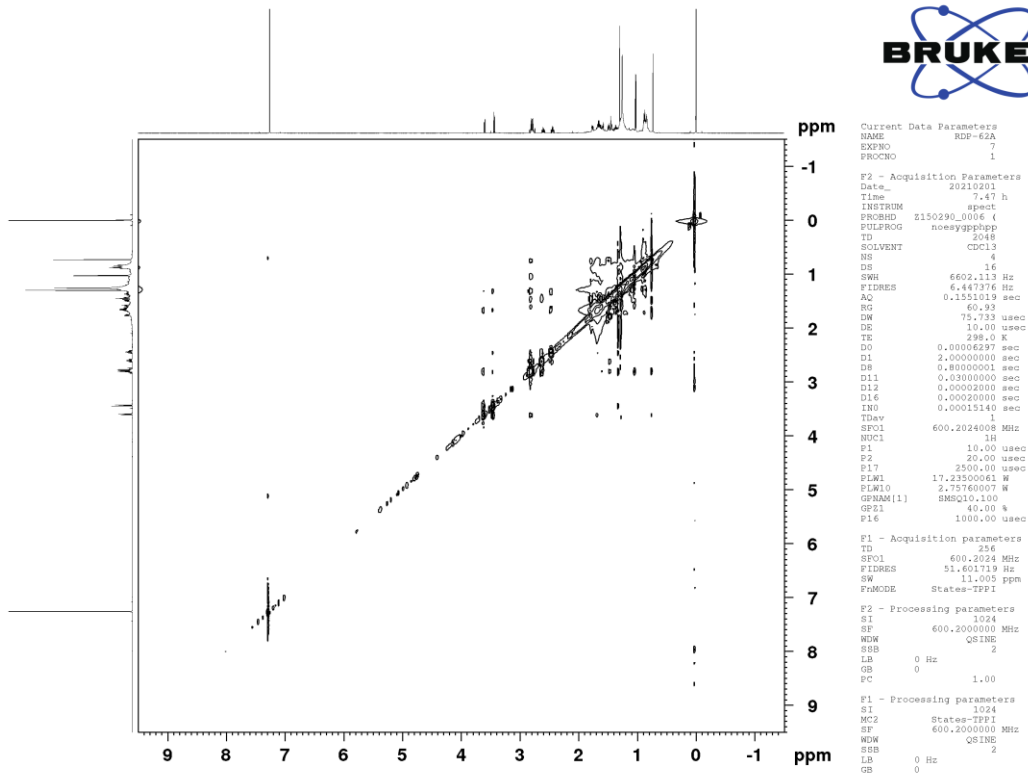


Figure S15 NOESY spectrum (600 MHz, CDCl₃) of compound 1

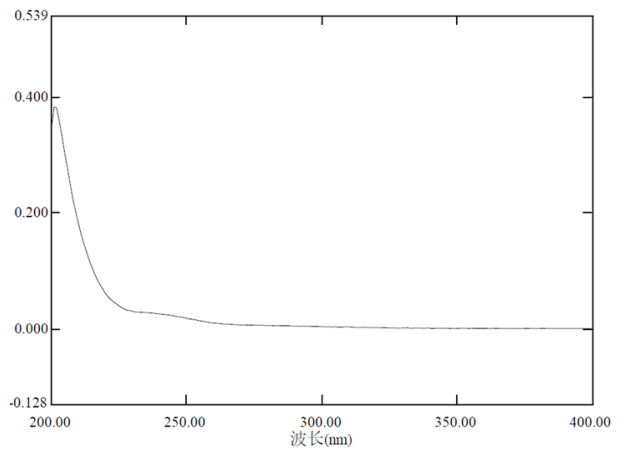
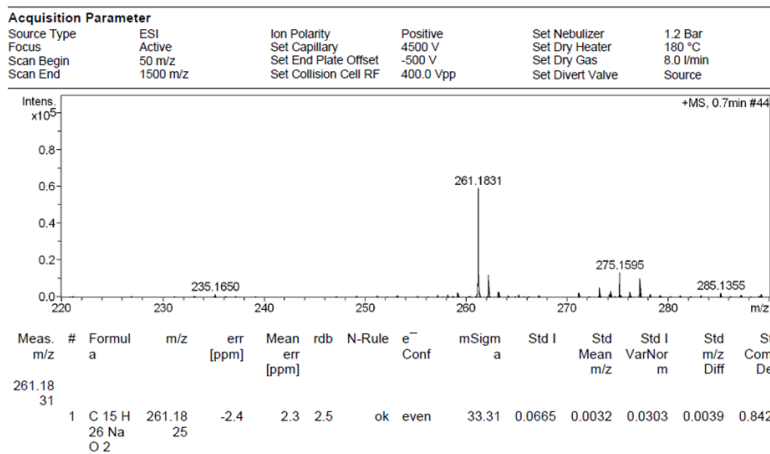
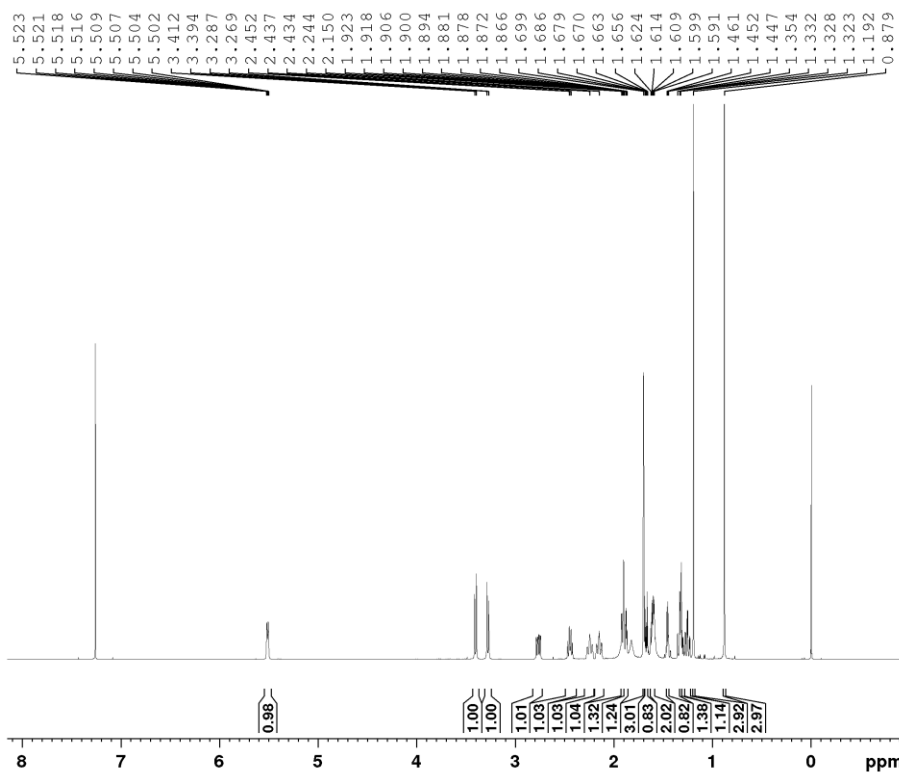


Figure S16 HRESIMS and UV spectra of compound 2



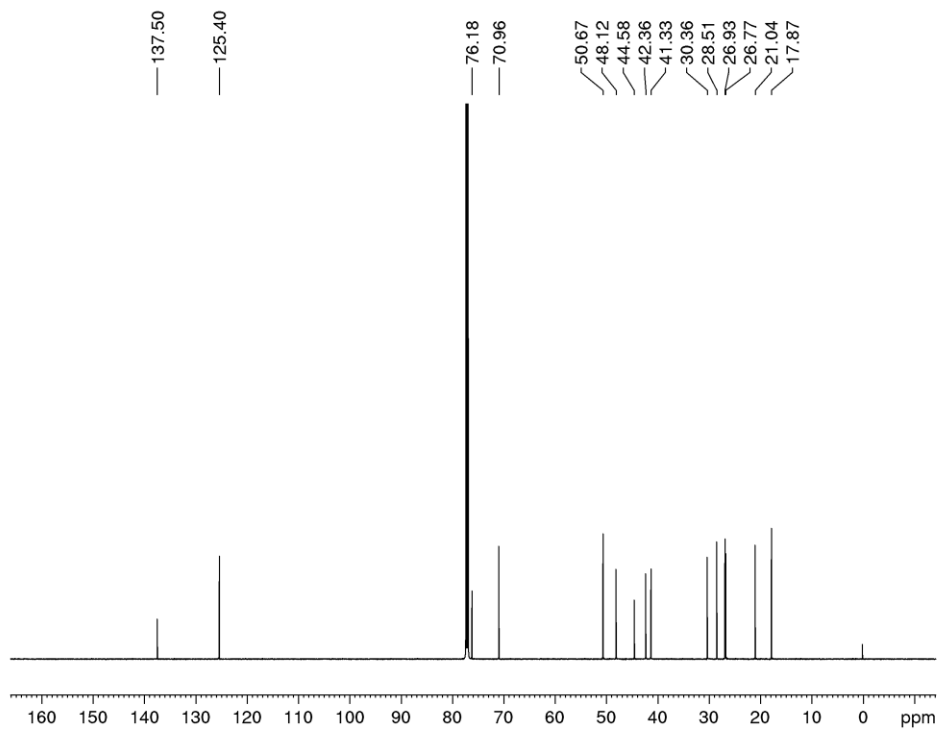
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EXPNO    10
PROCNO   1

F2 - Acquisition Parameters
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Time     14.42 h
INSTRUM  spect
PROBHD   Z150290_0006 (
PULPROG  zg30
TD       65536
SOLVENT  CDCl3
NS       2
DS       2
SWH      12019.230 Hz
FIDRES   0.366798 Hz
AQ       2.7262976 sec
RG       30.25
DW       41.600 usec
DE       10.00 usec
TE       298.0 K
D1       1.00000000 sec
TD0      1
SFO1     600.2037062 MHz
NUC1     1H
P0       3.33 usec
P1       10.00 usec
PLWL     17.23500061 W

F2 - Processing parameters
SI       65536
SF       600.2000137 MHz
WDW      EM
SSB      0
LB       0.30 Hz
GB       0
PC       1.00
  
```

Figure S17 ^1H NMR spectrum (600 MHz, CDCl_3) of compound 2



```

Current Data Parameters
NAME      RDP-95!  1
EXPNO    2
PROCNO   1

F2 - Acquisition Parameters
Date_    20200830
Time     15.31 h
INSTRUM  spect
PROBHD   Z150290_0006 (
PULPROG  zgpg30
TD       65366
SOLVENT  CDCl3
NS       1024
DS       4
SWH      42613.637 Hz
FIDRES   1.304047 Hz
AQ       0.7668437 sec
RG       37.95
DW       11.733 usec
DE       18.00 usec
TE       298.0 K
D1       2.00000000 sec
D11      0.03000000 sec
TD0      1
SFO1     150.9355021 MHz
NUC1     13C
P0       3.33 usec
P1       10.00 usec
PLW1     31.21999986 W
SFO2     600.2024008 MHz
NUC2     1H
CPDPRG2  waltz65
PCPD2    80.00 usec
PLW2     17.23500061 W
PLW12    0.25963911 W
PLW13    0.13013110 W

F2 - Processing parameters
SI       32768
SF       150.9203892 MHz
WDW      EM
SSB      0
LB       1.00 Hz
GB       0
PC       1.40
  
```

Figure S18 ^{13}C NMR spectrum (150 MHz, CDCl_3) of compound 2

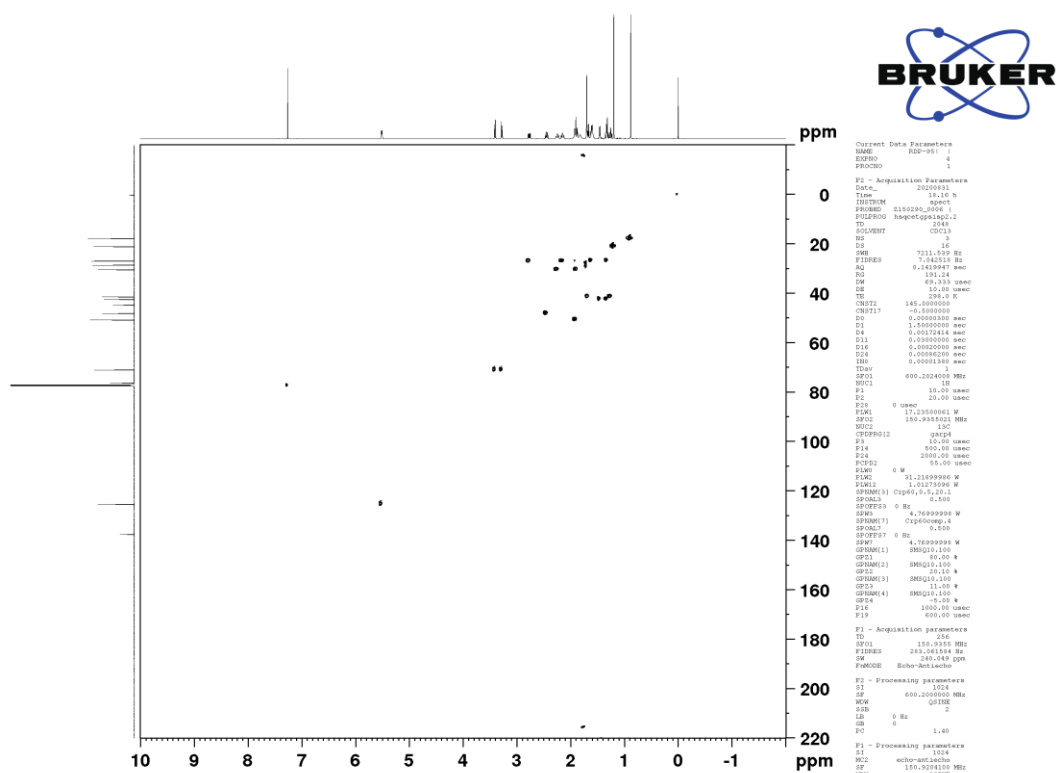


Figure S19 HSQC spectrum (600 MHz, CDCl₃) of compound 2

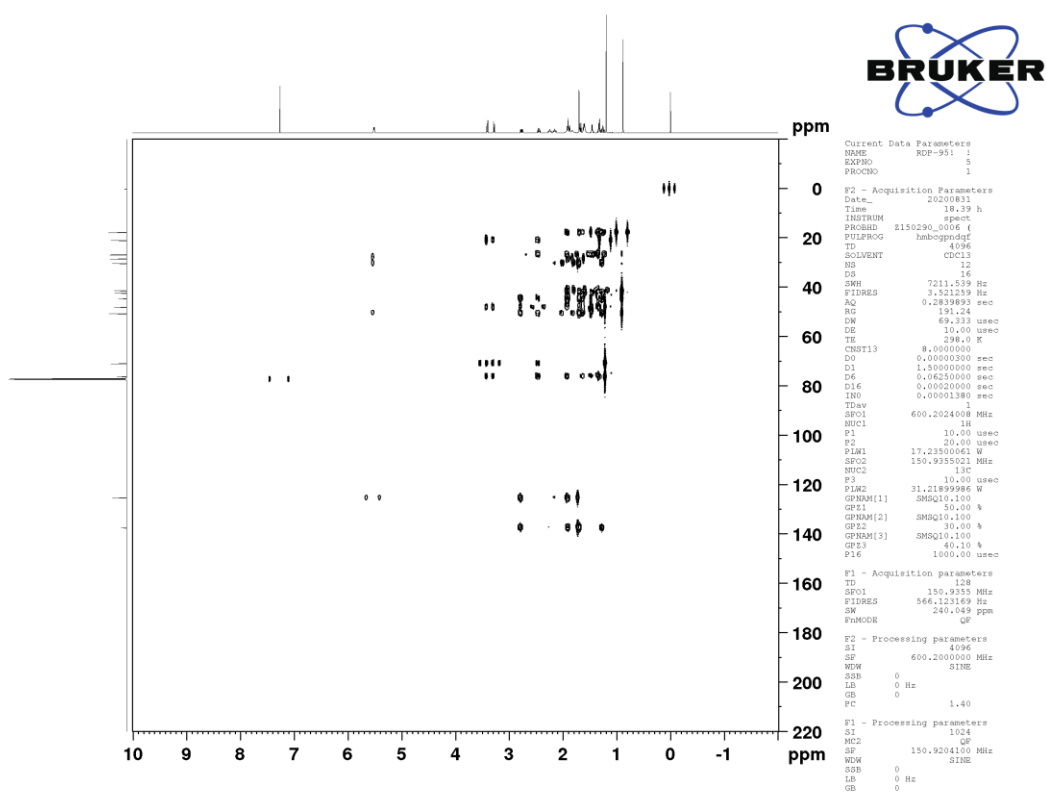


Figure S20 HMBC spectrum (600 MHz, CDCl₃) of compound 2

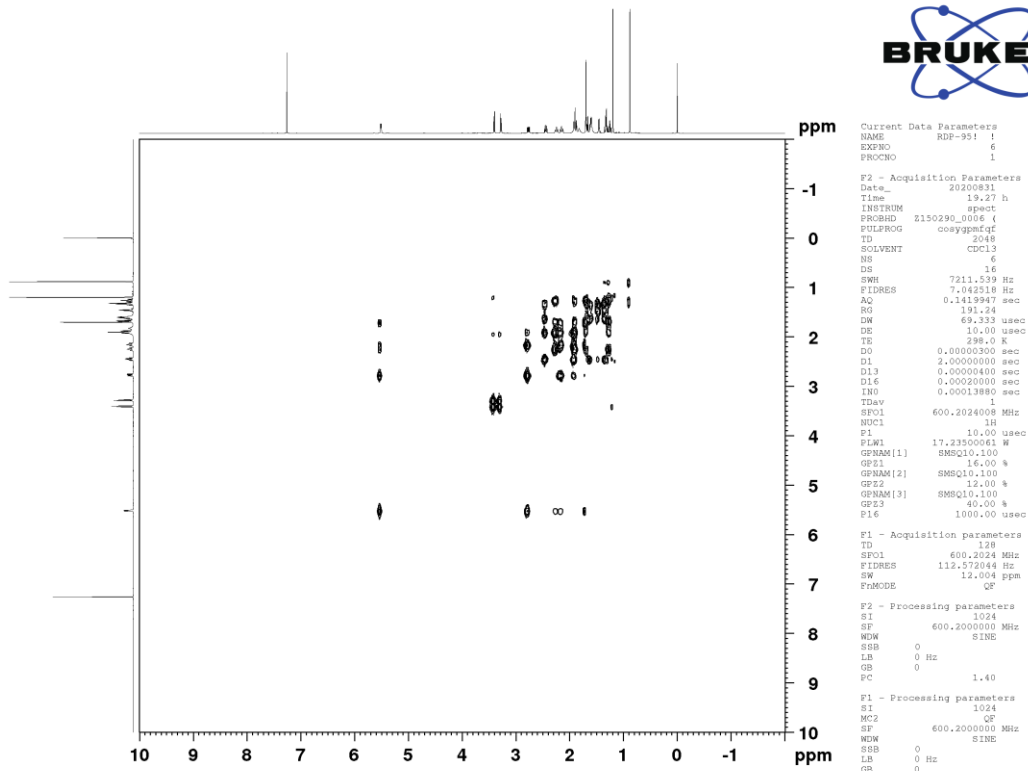


Figure S21 ^1H - ^1H COSY spectrum (600 MHz, CDCl_3) of compound 2

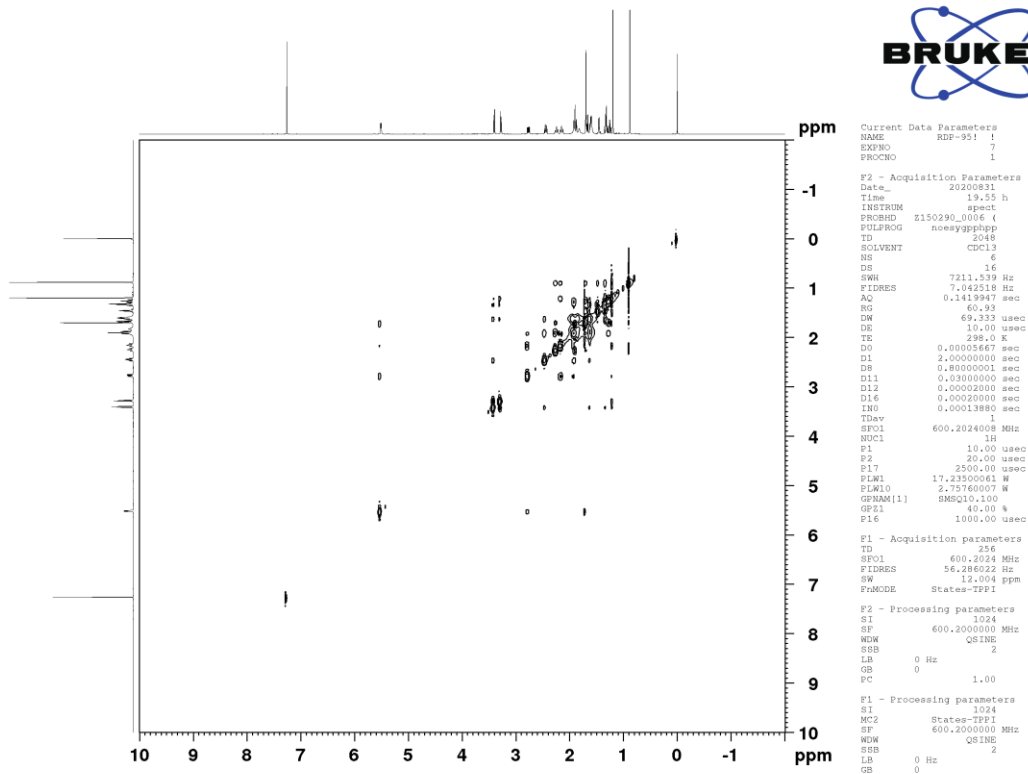


Figure S22 NOESY spectrum (600 MHz, CDCl_3) of compound 2

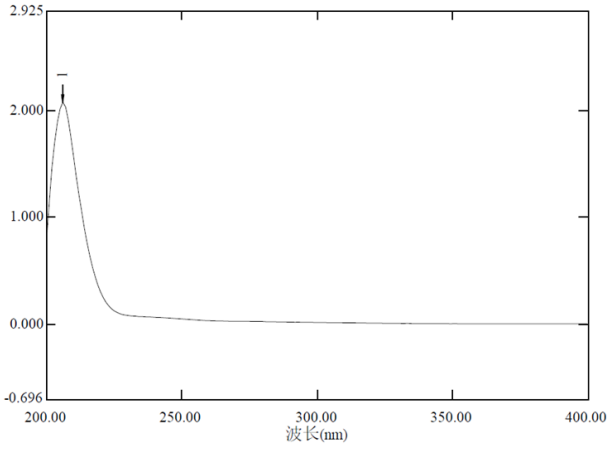
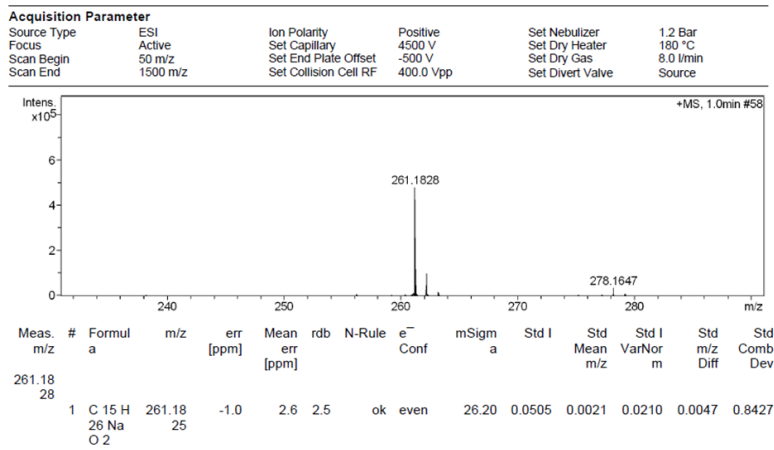


Figure S23 HRESIMS and UV spectra of compound **3**

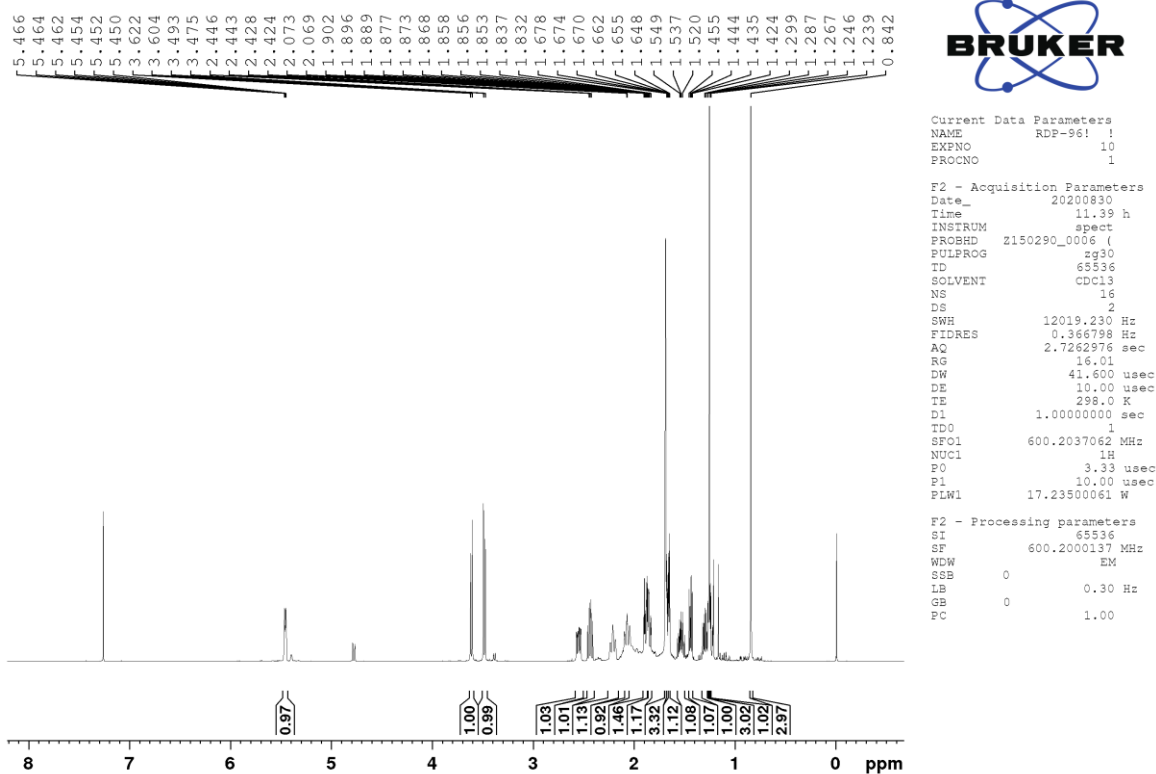


Figure S24 ¹H NMR spectrum (600 MHz, CDCl₃) of compound **3**

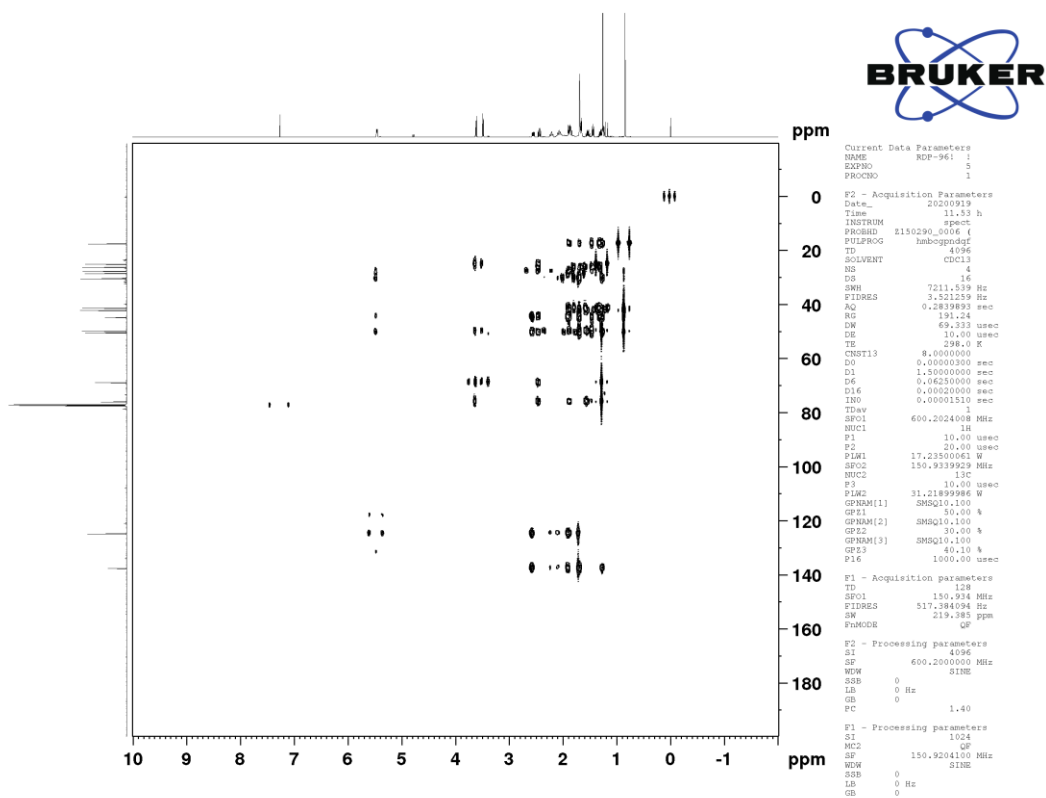


Figure S27 HMBC spectrum (600 MHz, CDCl₃) of compound 3

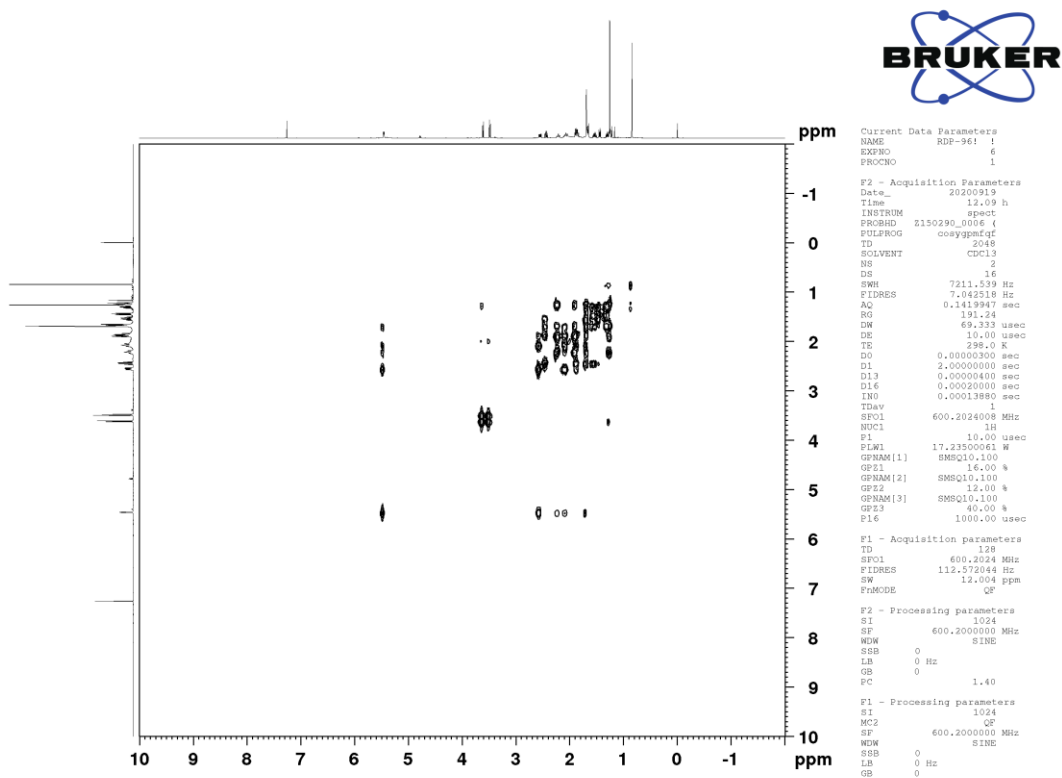


Figure S28 ¹H-¹H COSY spectrum (600 MHz, CDCl₃) of compound 3

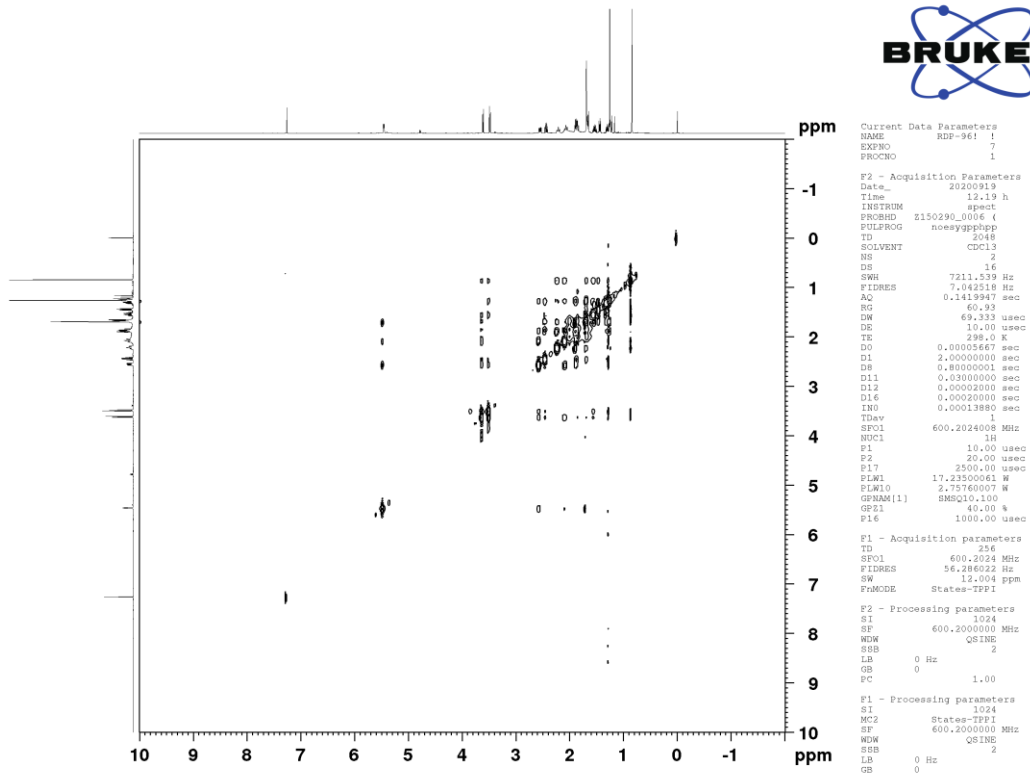


Figure S29 NOESY spectrum (600 MHz, CDCl₃) of compound 3

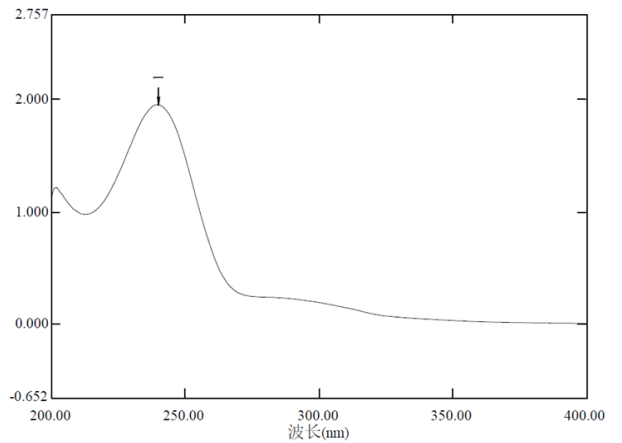
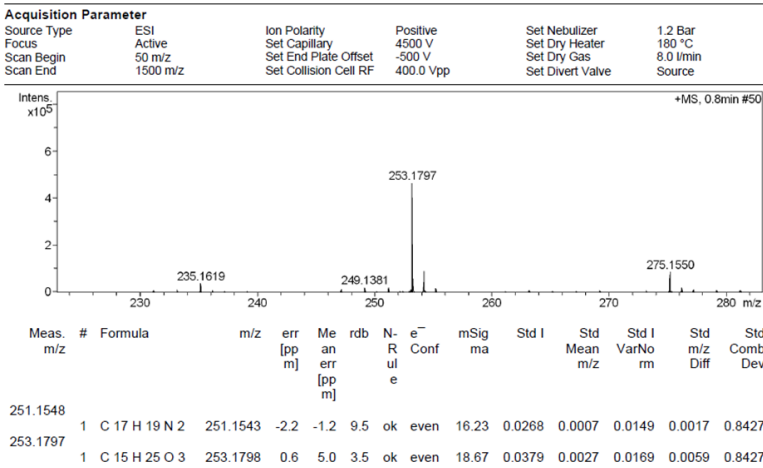


Figure S30 HRESIMS and UV spectra of compound 4

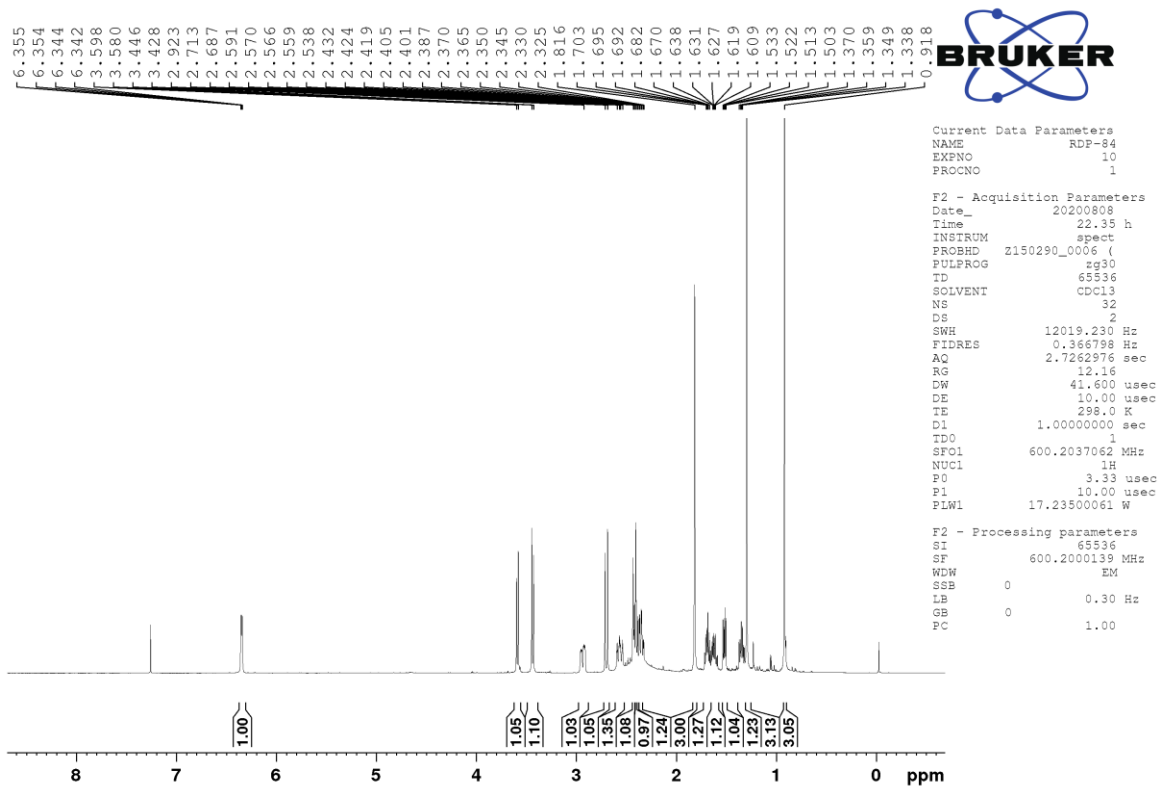


Figure S31 ^1H NMR spectrum (600 MHz, CDCl_3) of compound 4

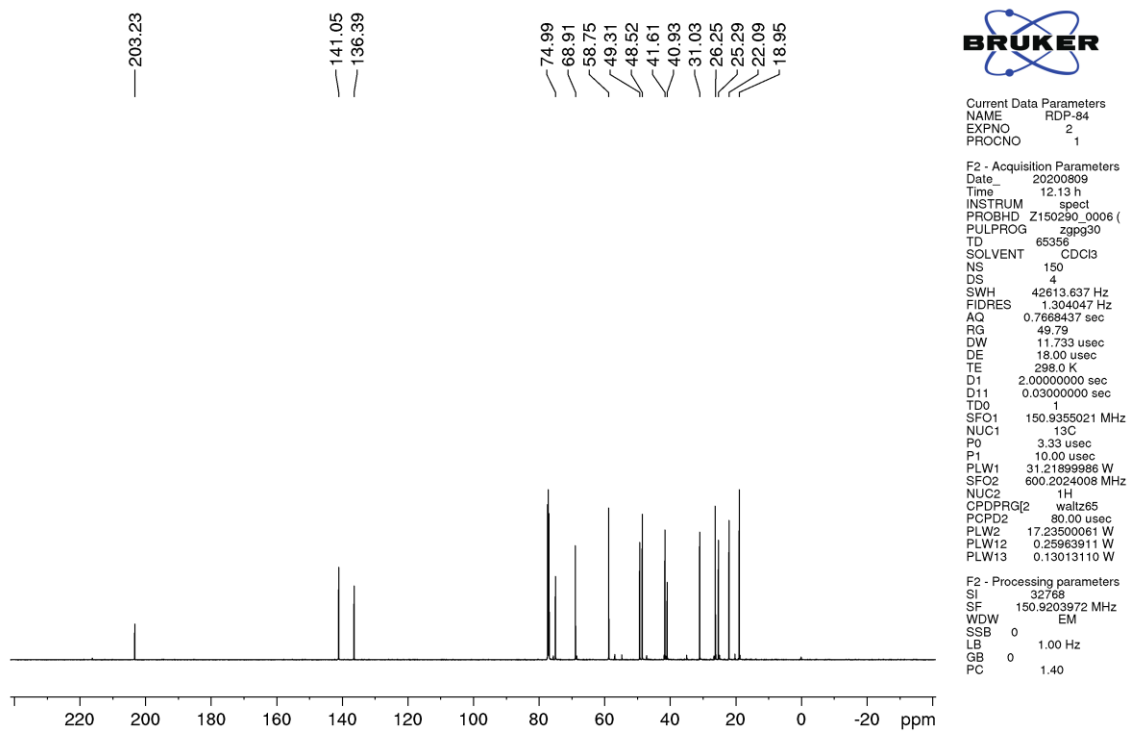


Figure S32 ^{13}C NMR spectrum (150 MHz, CDCl_3) of compound 4

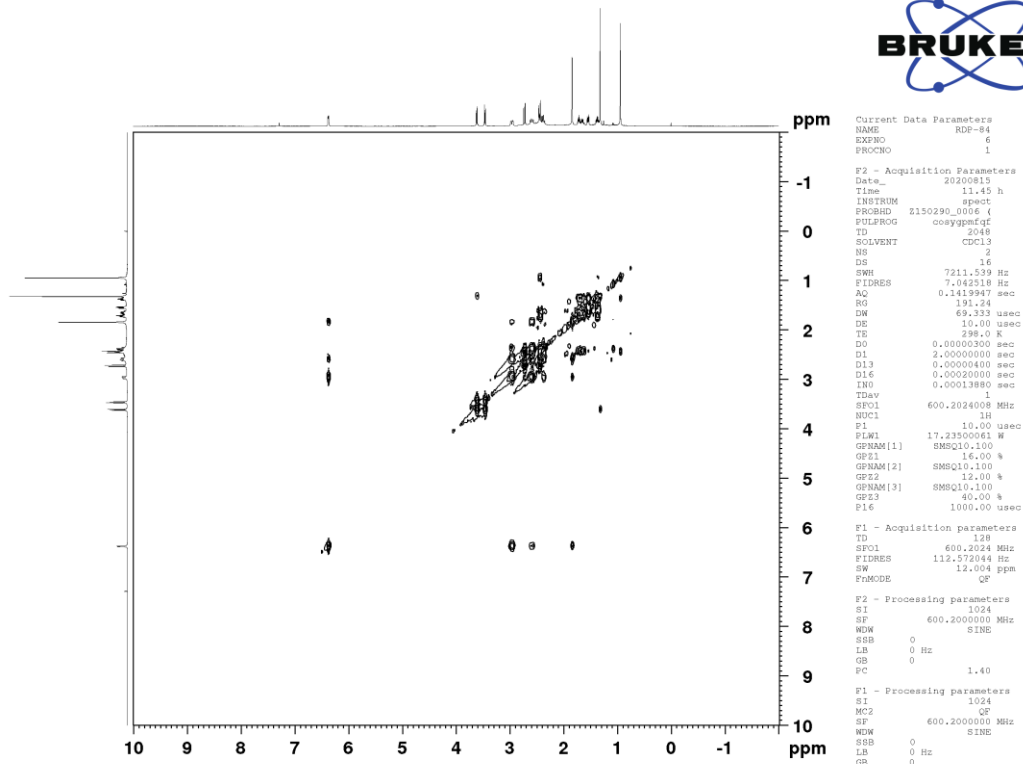


Figure S35 ^1H - ^1H COSY spectrum (600 MHz, CDCl_3) of compound 4

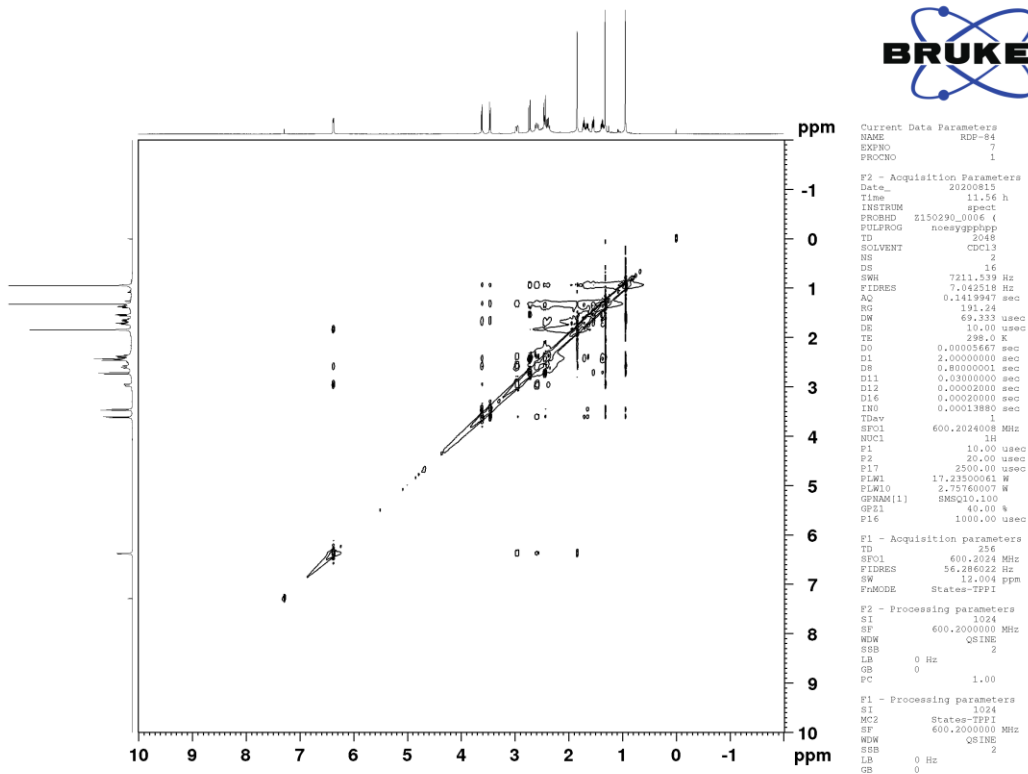


Figure S36 NOESY spectrum (600 MHz, CDCl_3) of compound 4

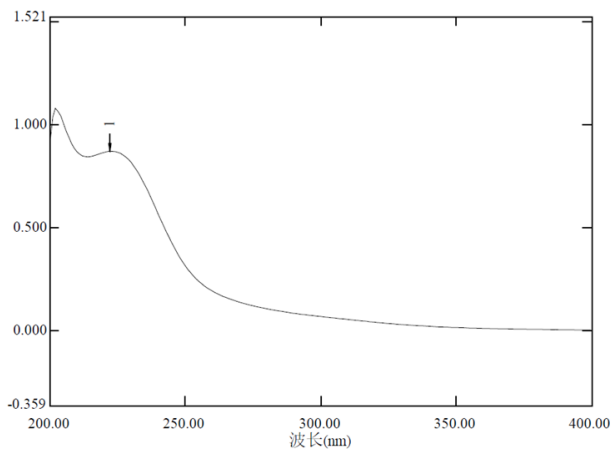
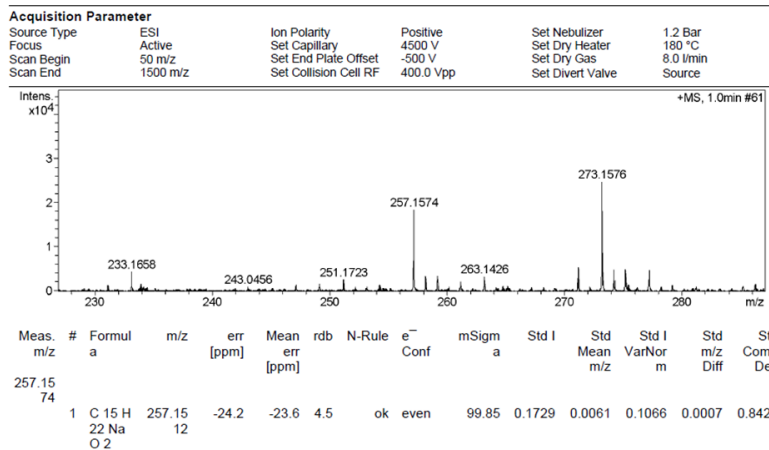
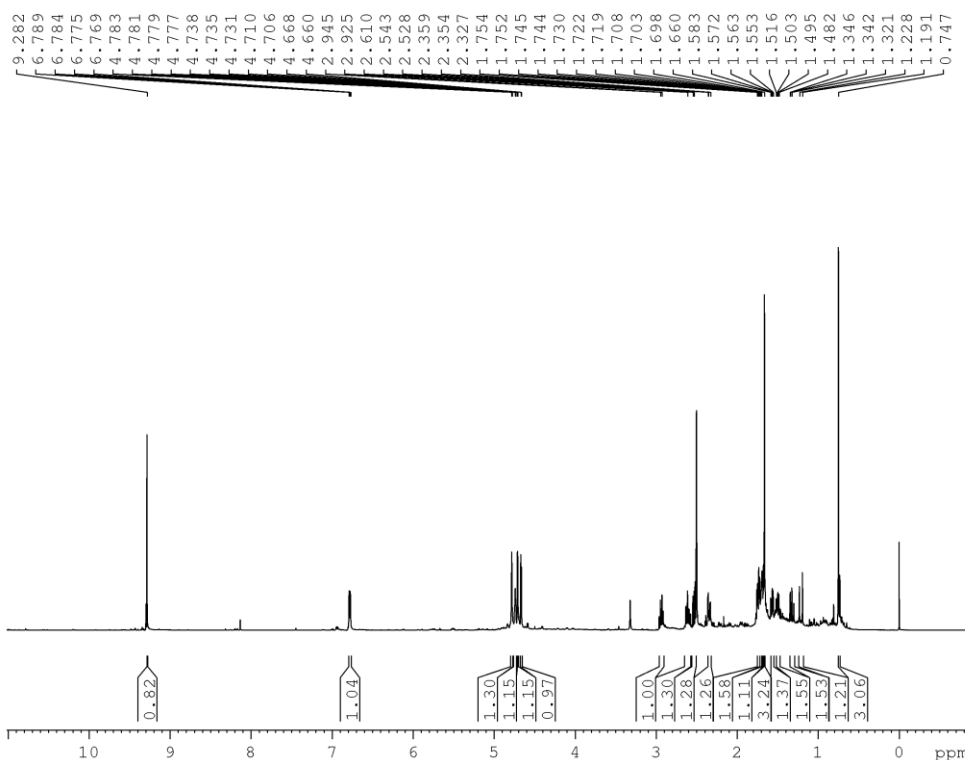


Figure S37 HRESIMS and UV spectra of compound **5**



Current Data Parameters
 NAME RDP-14!!
 EXPNO 1
 PROCNO 1

F2 - Acquisition Parameters
 Date_ 20200402
 Time 18:51 h
 INSTRUM spect
 PROBHD Z818801_0163 ()
 PULPROG zg30
 TD 65536
 SOLVENT DMSO
 NS 32
 DS 2
 SWH 12019.230 Hz
 FIDRES 0.366798 Hz
 AQ 2.7262976 sec
 RG 75.38
 DW 41.600 usec
 DE 6.50 usec
 TE 298.0 K
 D1 1.00000000 sec
 TD0 1
 SFO1 600.2037062 MHz
 NUC1 ¹H
 P0 4.00 usec
 P1 12.00 usec
 PLW1 5.44000006 W

F2 - Processing parameters
 SI 65536
 SF 600.2000044 MHz
 WDW EM
 SSB 0
 LB 0.30 Hz
 GB 0
 PC 1.00

Figure S38 ¹H NMR spectrum (600 MHz, CDCl₃) of compound **5**

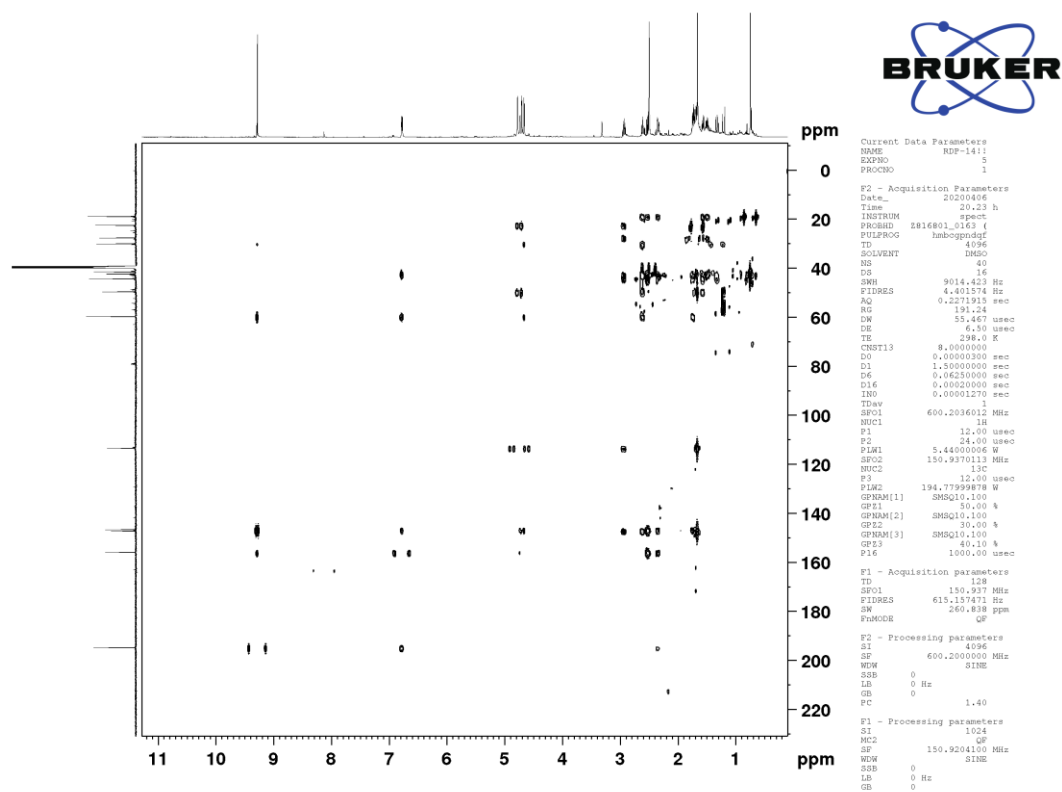


Figure S41 HMBC spectrum (600 MHz, CDCl₃) of compound 5

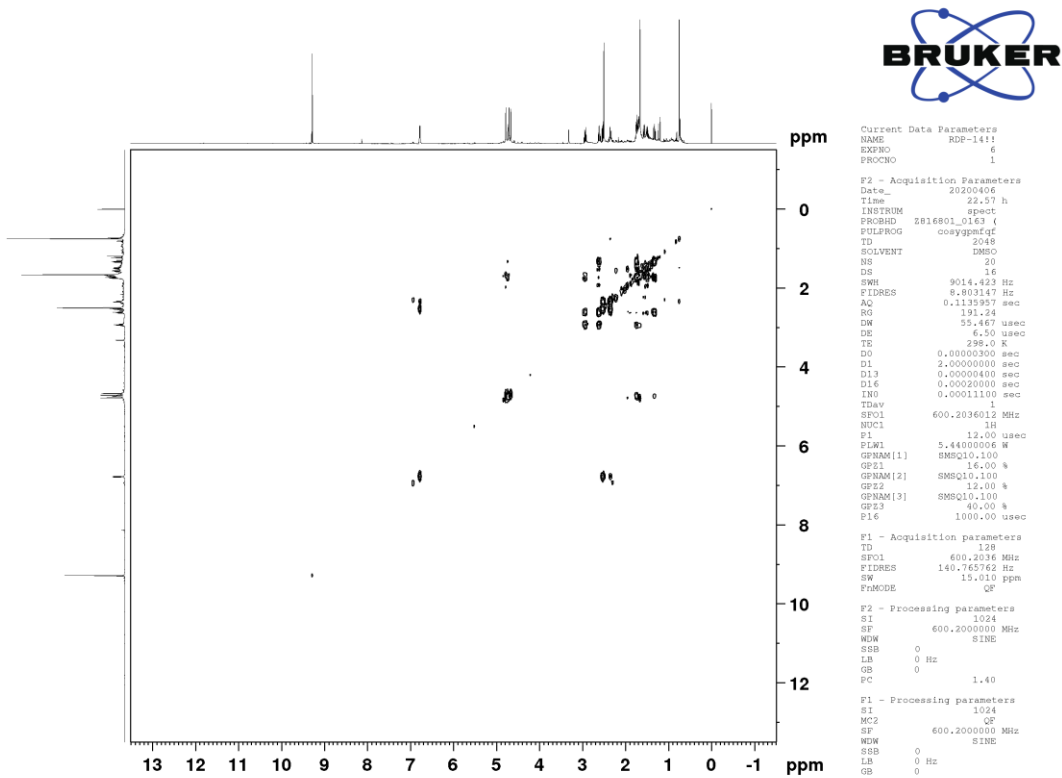


Figure S42 ¹H-¹H COSY spectrum (600 MHz, CDCl₃) of compound 5

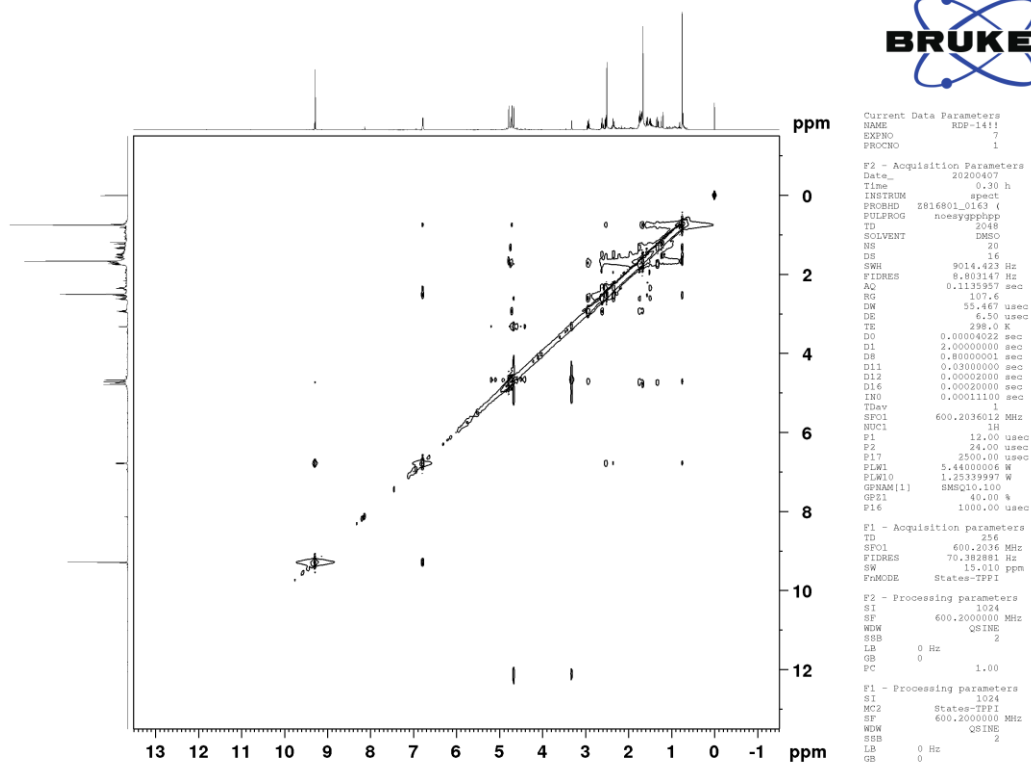


Figure S43 NOESY spectrum (600 MHz, CDCl₃) of compound 5

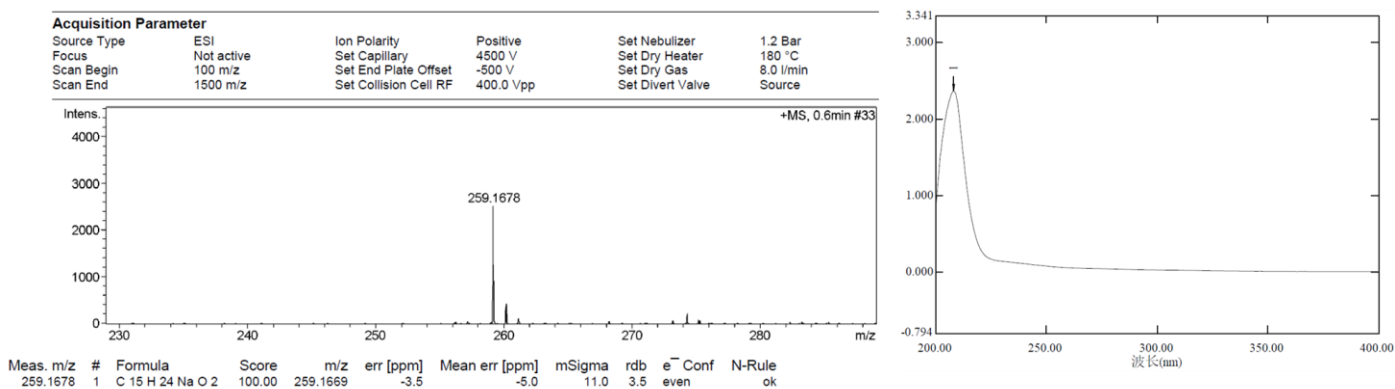
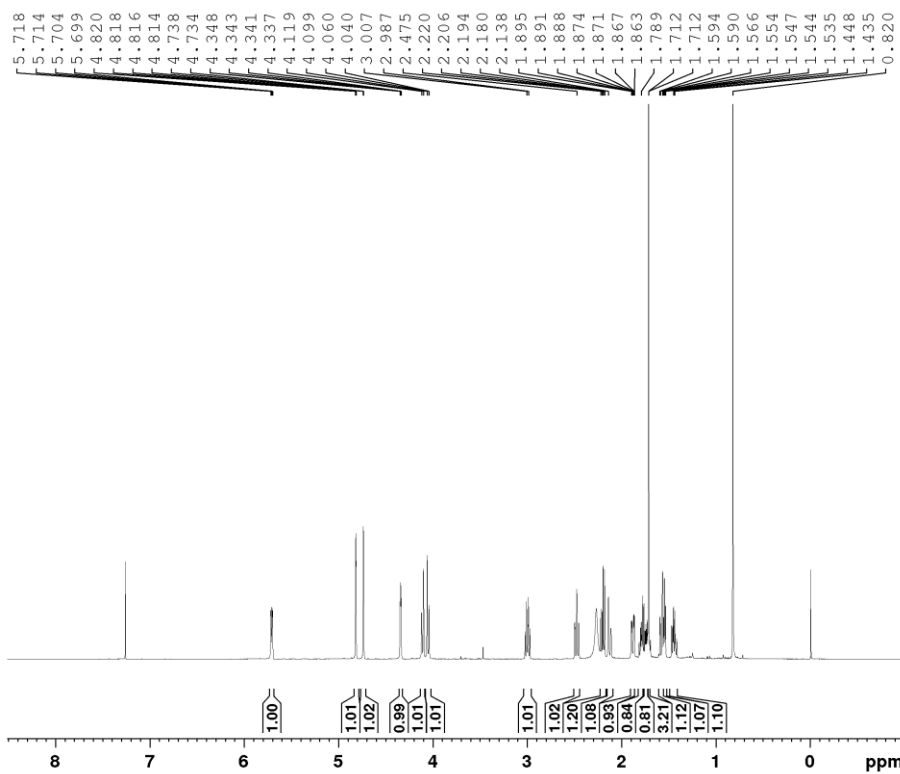


Figure S44 HRESIMS and UV spectra of compound 6



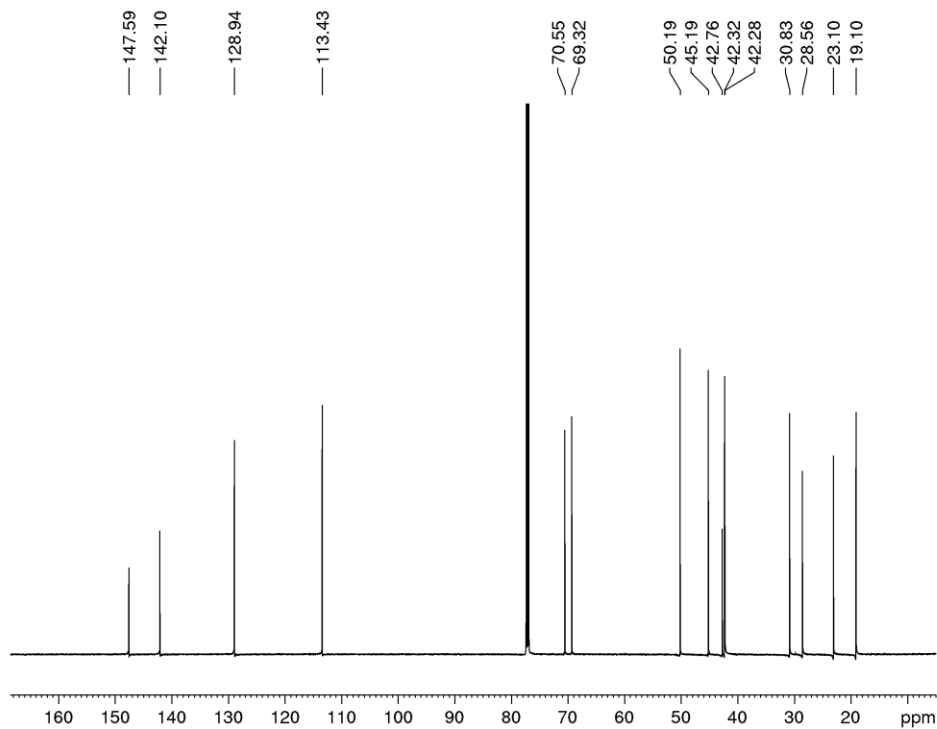
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Current Data Parameters
NAME      RDP-123B
EXPNO    10
PROCNO   1

F2 - Acquisition Parameters
Date_    20201027
Time     23.51 h
INSTRUM  spect
PROBHD   Z150290_0006 (
PULPROG  zg30
TD       65536
SOLVENT  CDCl3
NS       16
DS       2
SWH      12019.230 Hz
FIDRES   0.366798 Hz
AQ       2.7262976 sec
RG       12.16
DW       41.600 usec
DE       10.00 usec
TE       298.0 K
D1       1.00000000 sec
TD0      1
SFO1     600.2037062 MHz
NUC1     1H
P0       3.33 usec
P1       10.00 usec
PLWL     17.23500061 W

F2 - Processing parameters
SI       65536
SF       600.2000136 MHz
WDW      EM
SSB      0
LB       0.30 Hz
GB       0
PC       1.00
  
```

Figure S45 ^1H NMR spectrum (600 MHz, CDCl_3) of compound 6



```

Current Data Parameters
NAME      RDP-123B
EXPNO    2
PROCNO   1

F2 - Acquisition Parameters
Date_    20201028
Time     0.01 h
INSTRUM  spect
PROBHD   Z150290_0006 (
PULPROG  zgpg30
TD       65536
SOLVENT  CDCl3
NS       200
DS       4
SWH      42613.637 Hz
FIDRES   1.300465 Hz
AQ       0.7689557 sec
RG       24.61
DW       11.733 usec
DE       18.00 usec
TE       298.0 K
D1       2.00000000 sec
D11      0.03000000 sec
TD0      1
SFO1     150.9355021 MHz
NUC1     13C
P0       3.33 usec
P1       10.00 usec
PLW1     31.21999986 W
SFO2     600.2024008 MHz
NUC2     1H
CPDPRG2  waltz65
PCPD2    80.00 usec
PLW2     17.23500061 W
PLW12    0.25963911 W
PLW13    0.13013110 W

F2 - Processing parameters
SI       32768
SF       150.9203924 MHz
WDW      EM
SSB      0
LB       1.00 Hz
GB       0
PC       1.40
  
```

Figure S46 ^{13}C NMR spectrum (150 MHz, CDCl_3) of compound 6

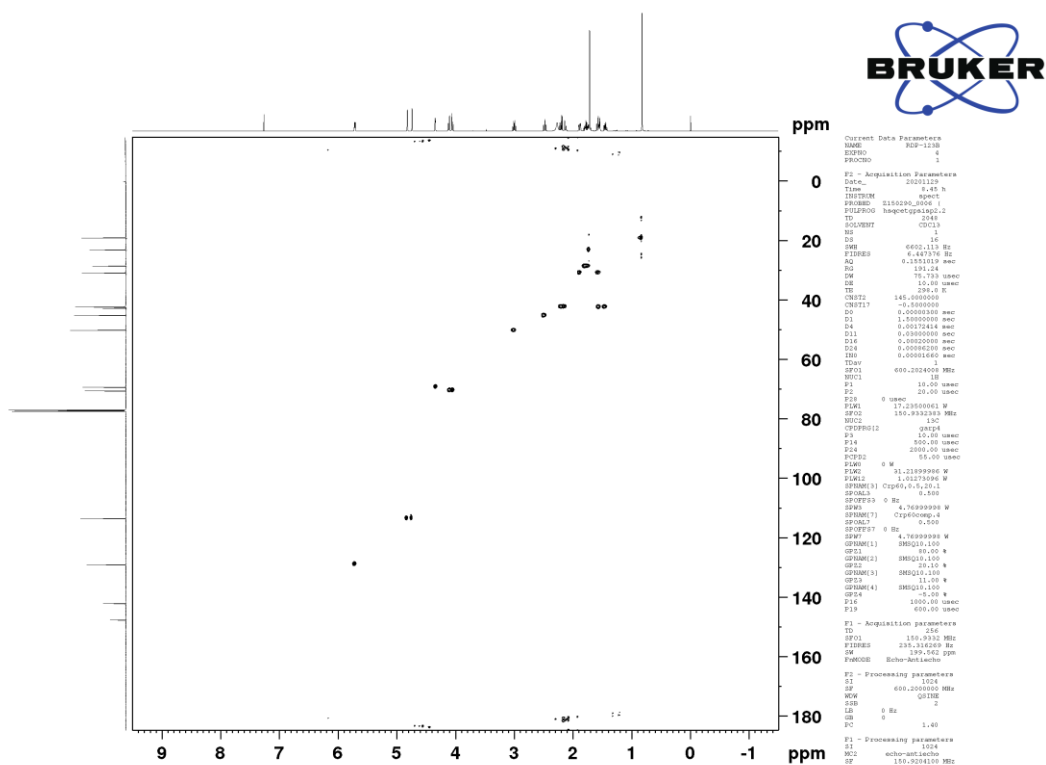


Figure S47 HSQC spectrum (600 MHz, CDCl₃) of compound 6

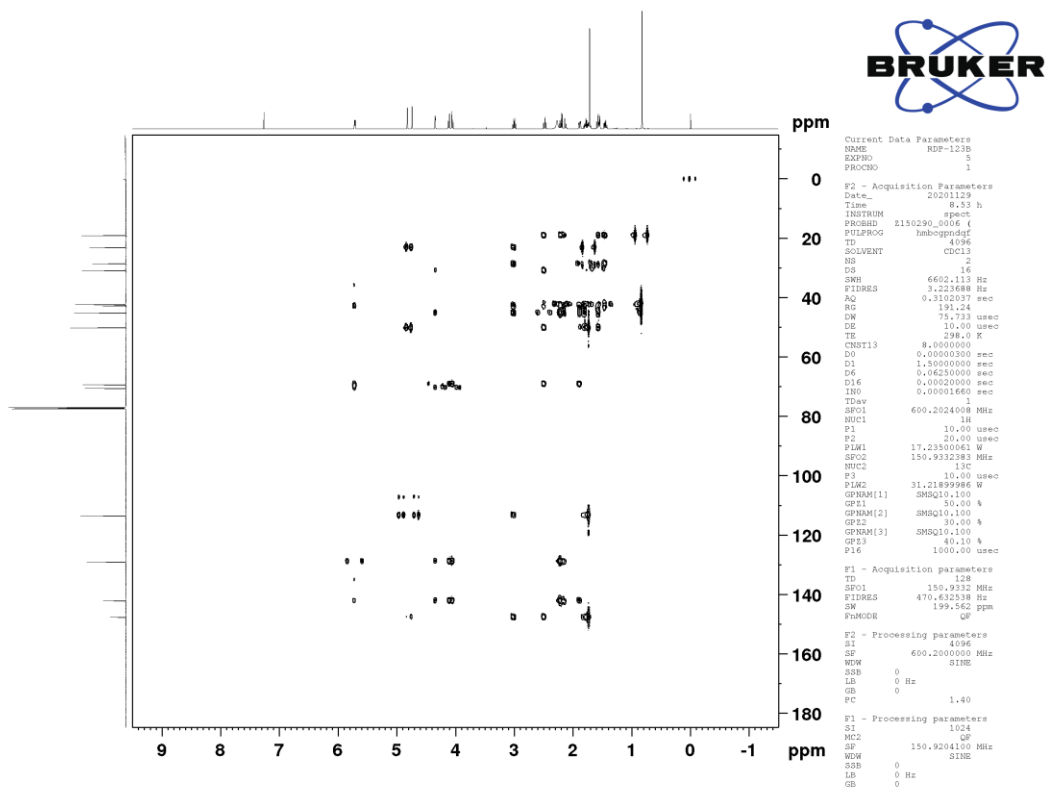


Figure S48 HMBC spectrum (600 MHz, CDCl₃) of compound 6

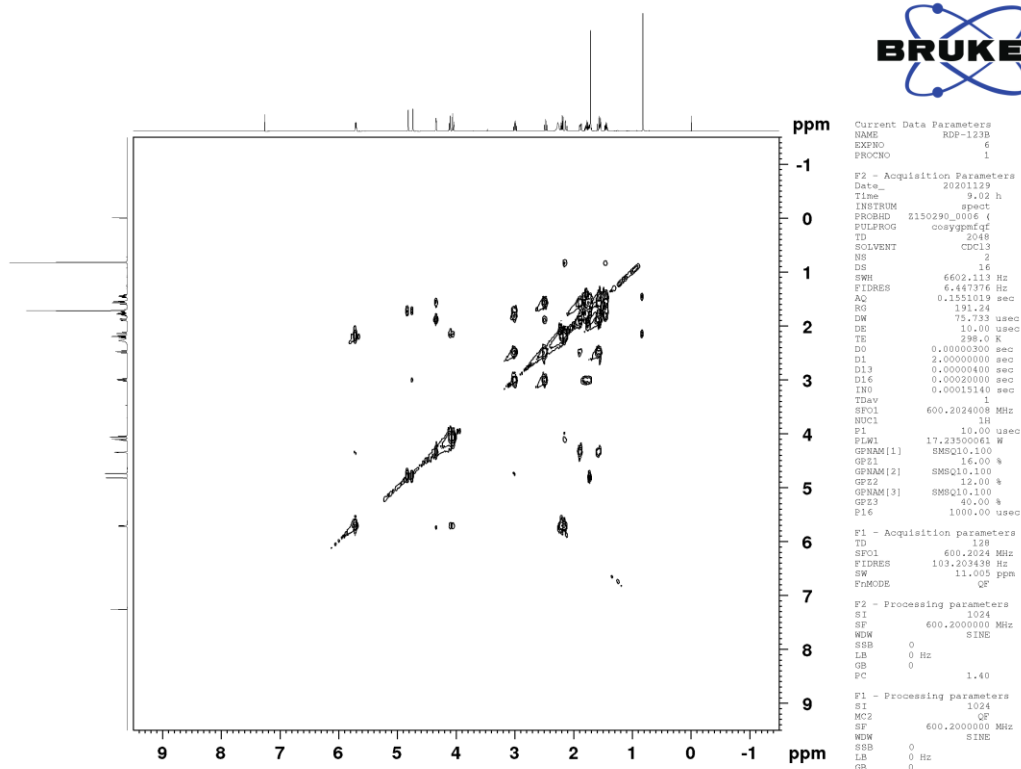


Figure S49 ^1H - ^1H COSY spectrum (600 MHz, CDCl_3) of compound 6

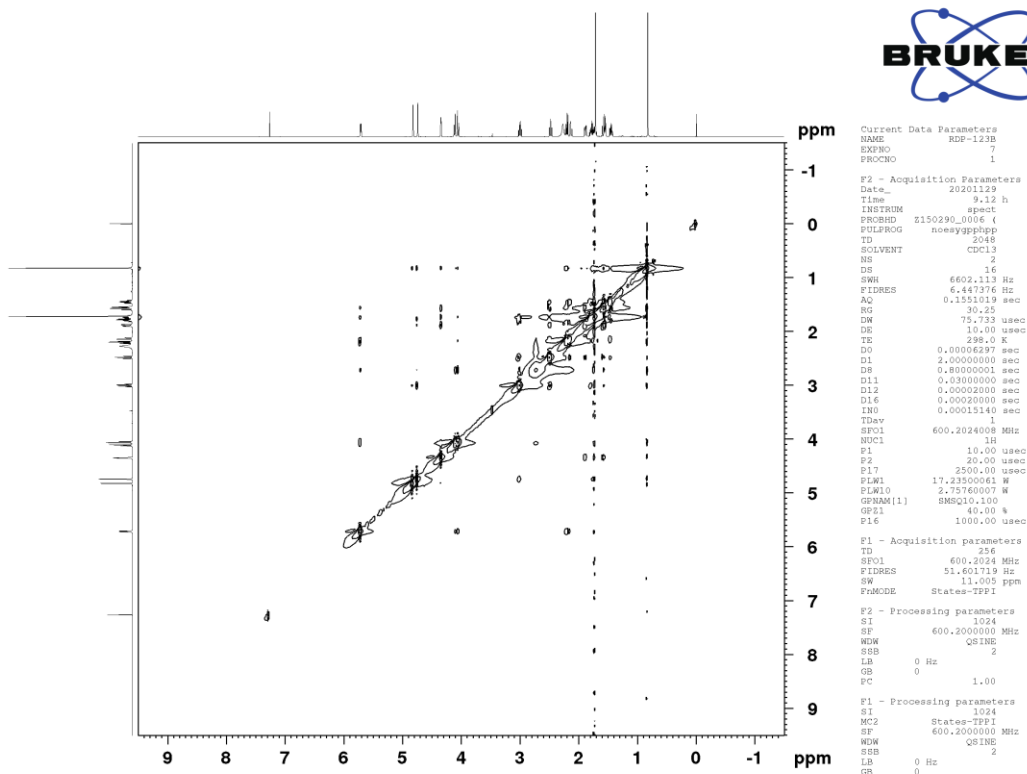


Figure S50 NOESY spectrum (600 MHz, CDCl_3) of compound 6

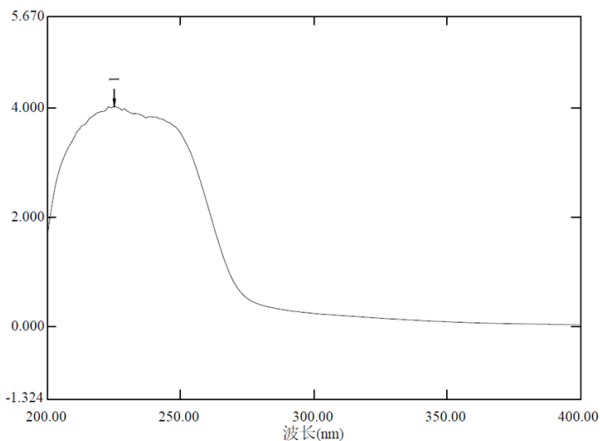
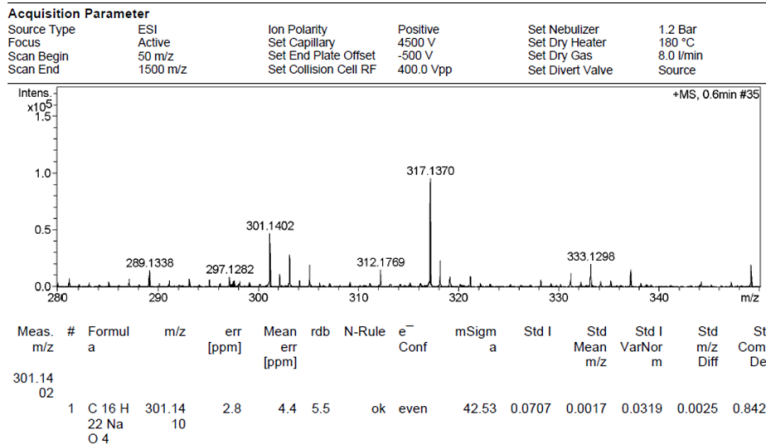


Figure S51 HRESIMS and UV spectra of compound **7**

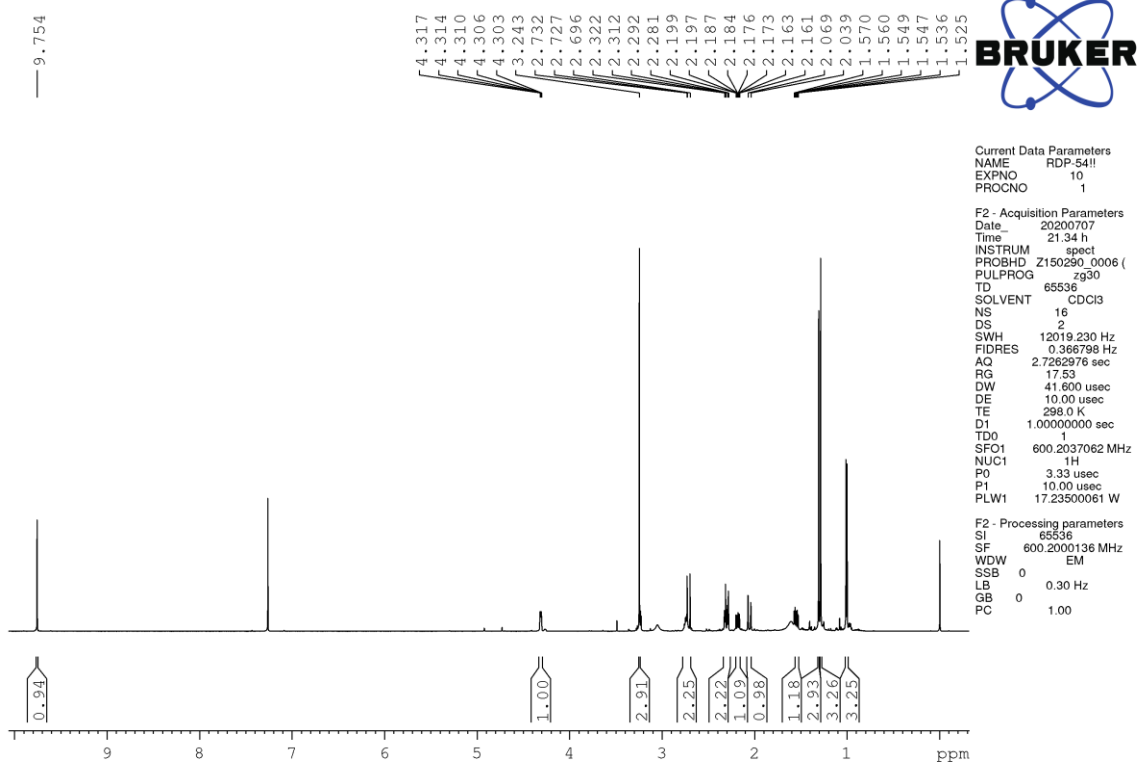


Figure S52 ¹H NMR spectrum (600 MHz, CDCl₃) of compound **7**

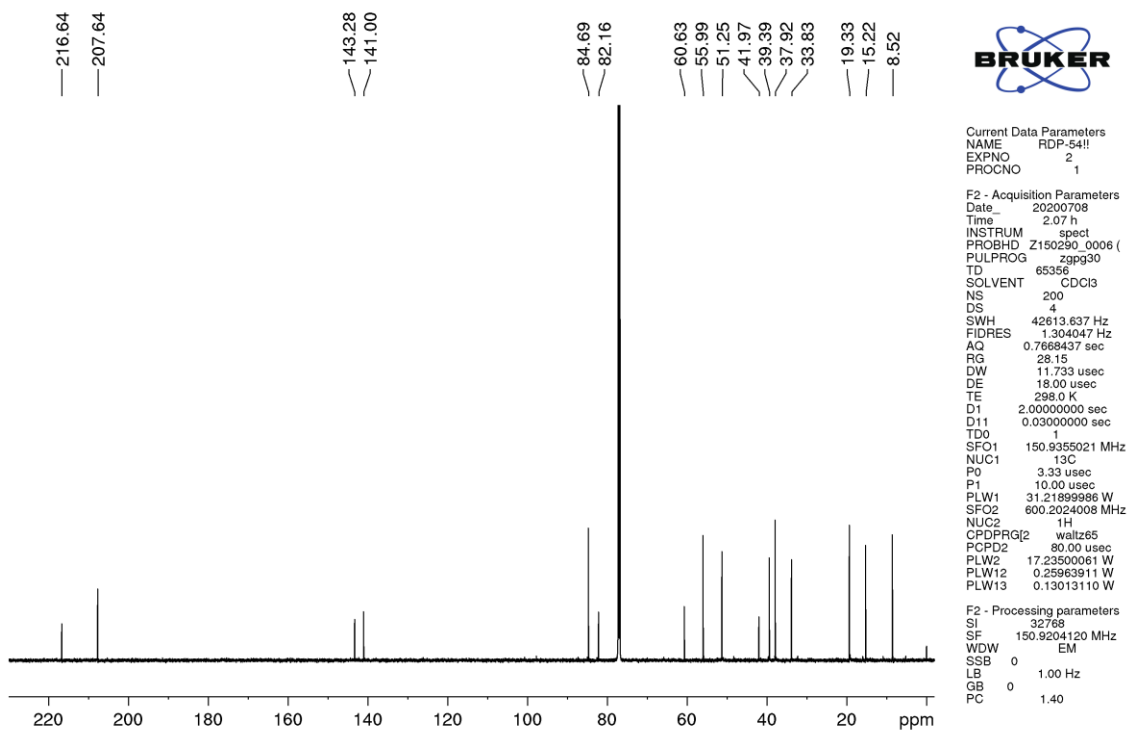


Figure S53 ^{13}C NMR spectrum (150 MHz, CDCl_3) of compound 7

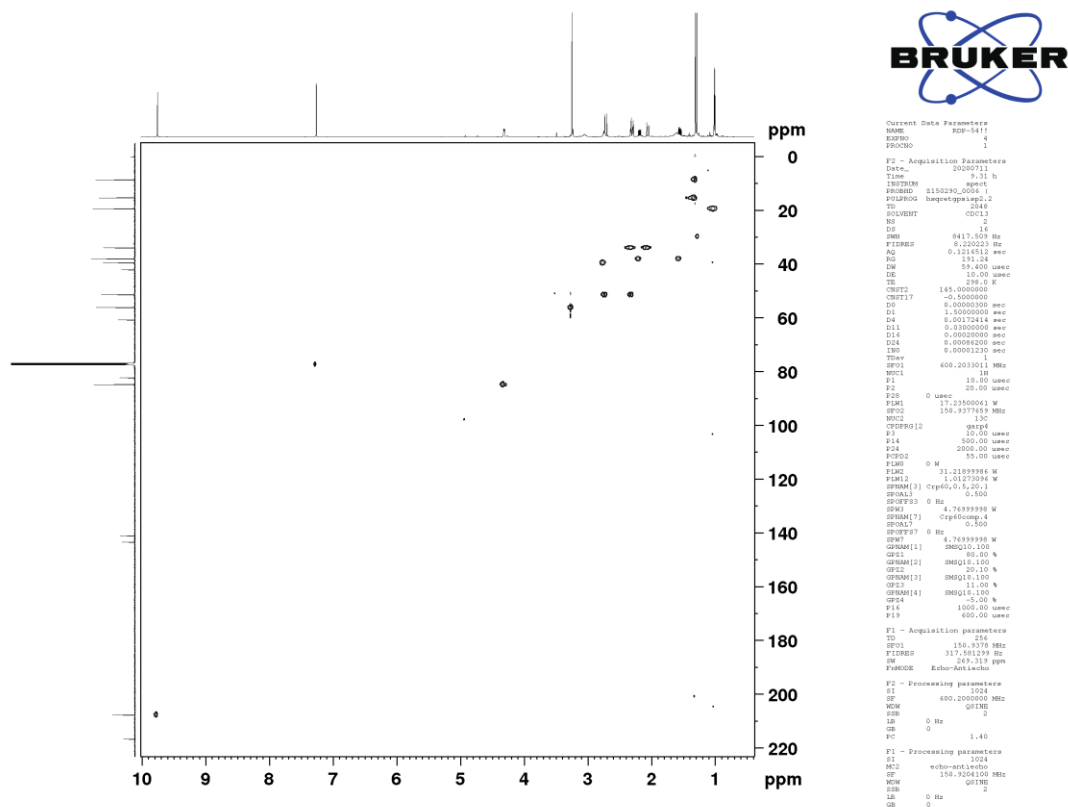


Figure S54 HSQC spectrum (600 MHz, CDCl_3) of compound 7

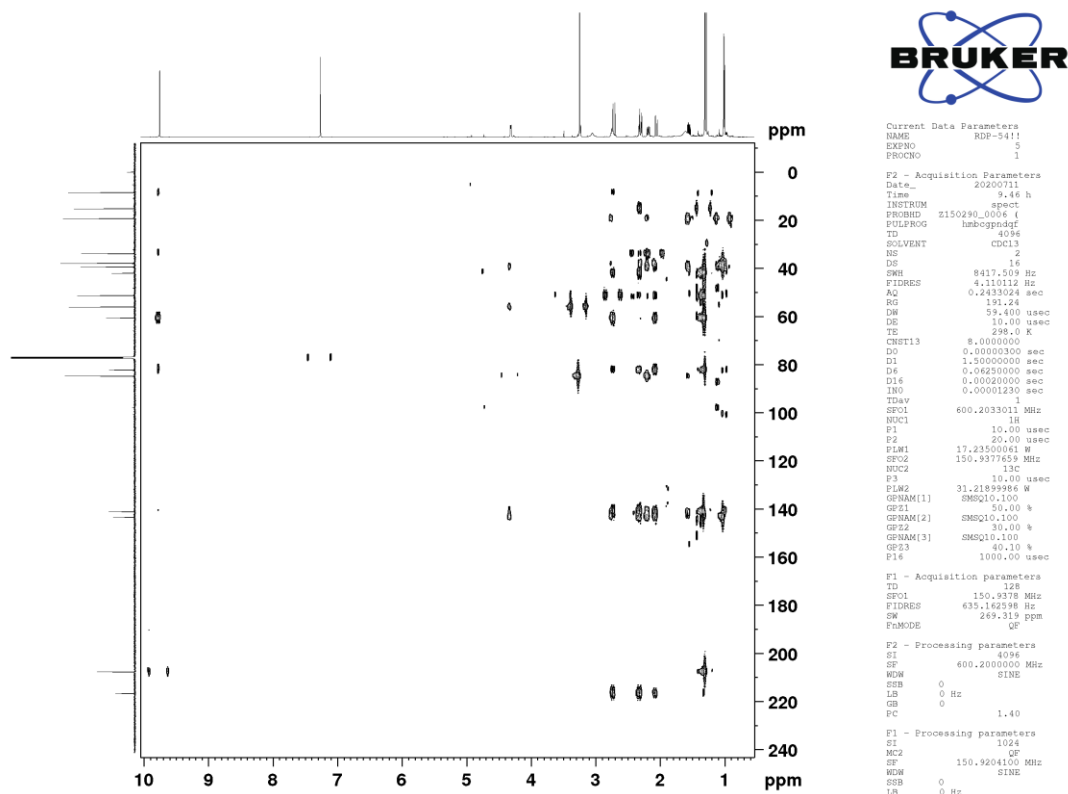


Figure S55 HMBC spectrum (600 MHz, CDCl₃) of compound 7

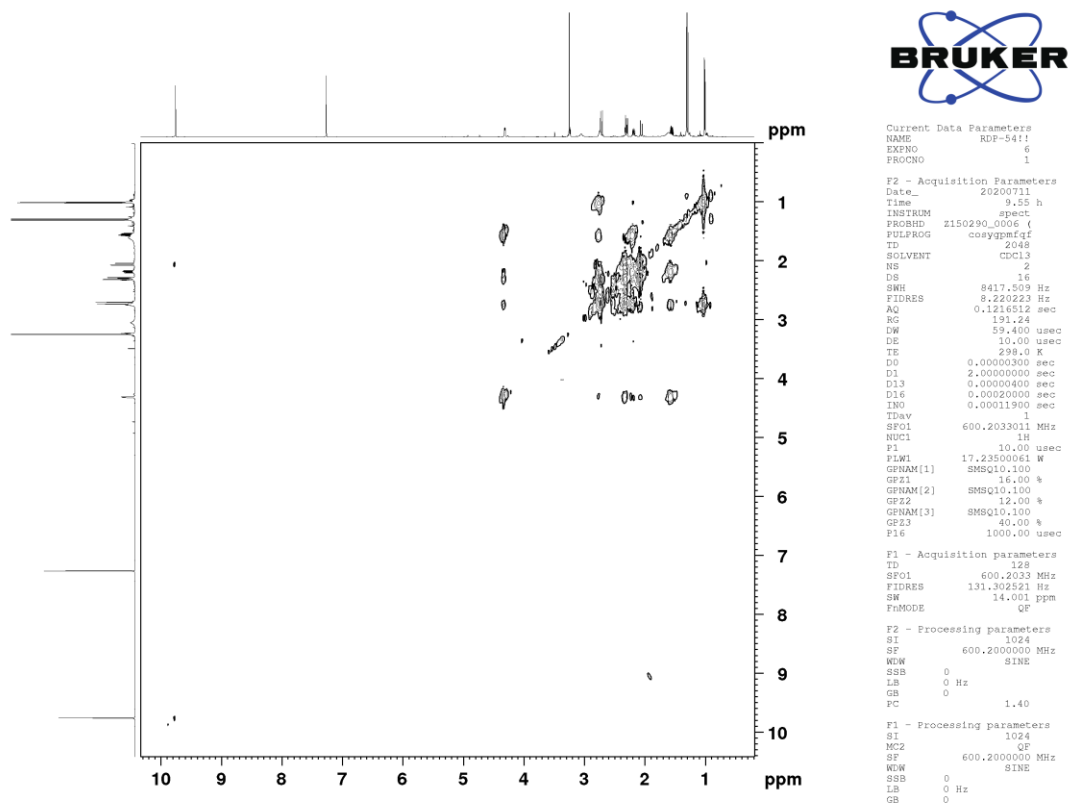
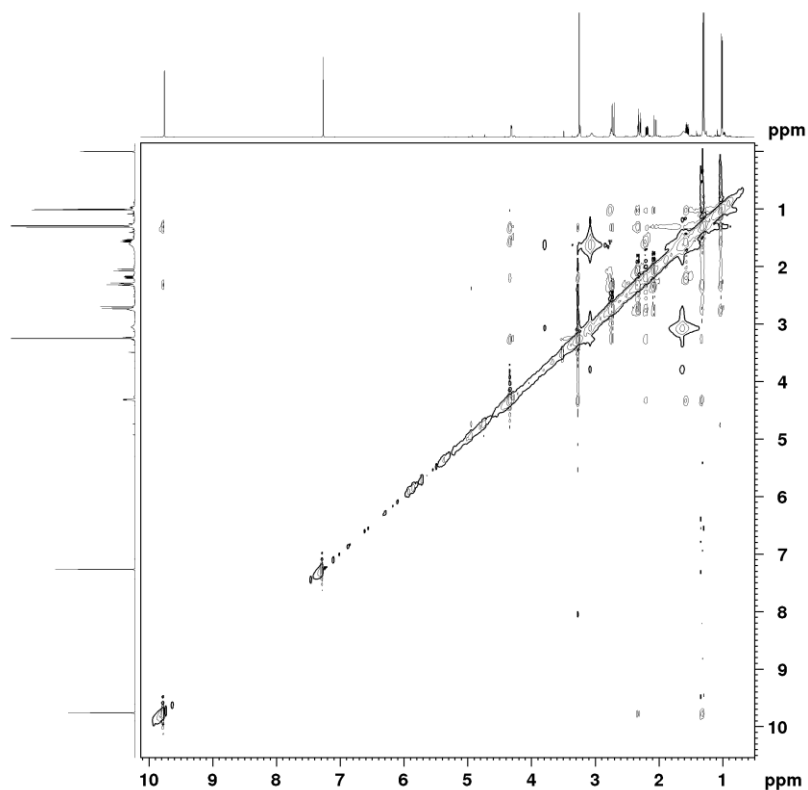


Figure S56 ¹H-¹H COSY spectrum (600 MHz, CDCl₃) of compound 7



```
Current Data Parameters
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EXNO     7
PROCNO   1

F2 - Acquisition Parameters
Date_    20200711
Time     10.06 h
INSTRUM  spect
PROBHD   Z150290_0006 (
PULPROG  ncesypphpgp
TE       2048
SOLVENT  CDCl3
NS       2
DS       16
SWH      8417.509 Hz
FIDRES   8.220223 Hz
AQ       0.1216512 sec
RG       60.93
DW       59.400 usec
DE       10.00 usec
TE       298.0 K
D0       0.00004677 sec
D1       2.00000000 sec
D8       0.80000001 sec
D11      0.03000000 sec
D12      0.00002000 sec
D16      0.00020000 sec
INO      0.00011900 sec
TDav     1
SF01     600.2033011 MHz
NUC1     13
P1       10.00 usec
P2       20.00 usec
P17     2500.00 usec
PLM1     17.23500041 W
PLM10    2.75760007 W
GRNAM(1) SMS901.100
GPZ1     40.00 %
P16     1000.00 usec

F1 - Acquisition parameters
TD       256
SF01     600.2033 MHz
FIDRES   65.651260 Hz
SW       14.001 ppm
P2MODE   States-TPPI

F2 - Processing parameters
SI       1024
SF       600.2000000 MHz
WDW      QSINE
SSB      2
LB       0 Hz
GB       0
PC       1.00

F1 - Processing parameters
SI       1024
MC2     States-TPPI
SF       600.2000000 MHz
WDW      QSINE
SSB      2
LB       0 Hz
GB       0
```

Figure S57 NOESY spectrum (600 MHz, CDCl₃) of compound 7

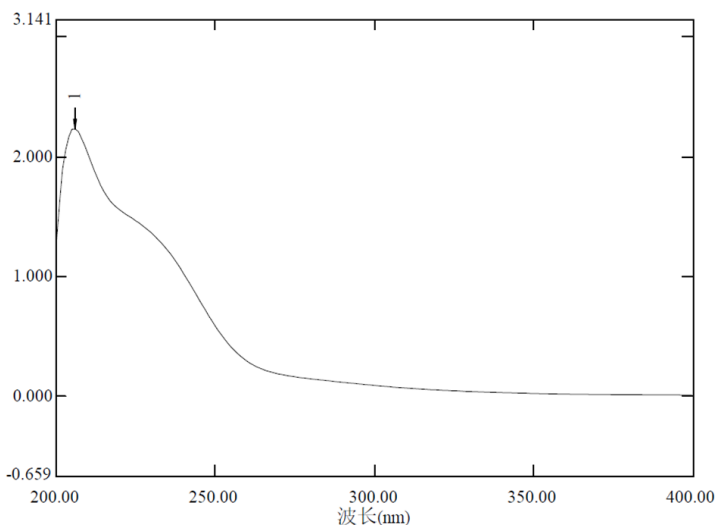
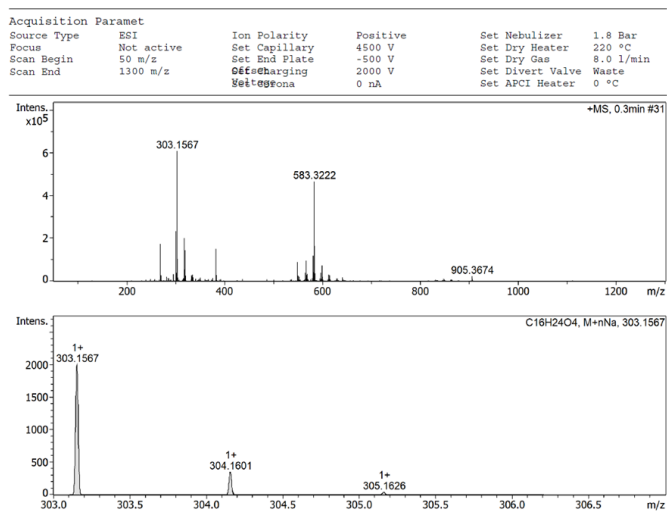


Figure S58 HRESIMS and UV spectra of compound 8

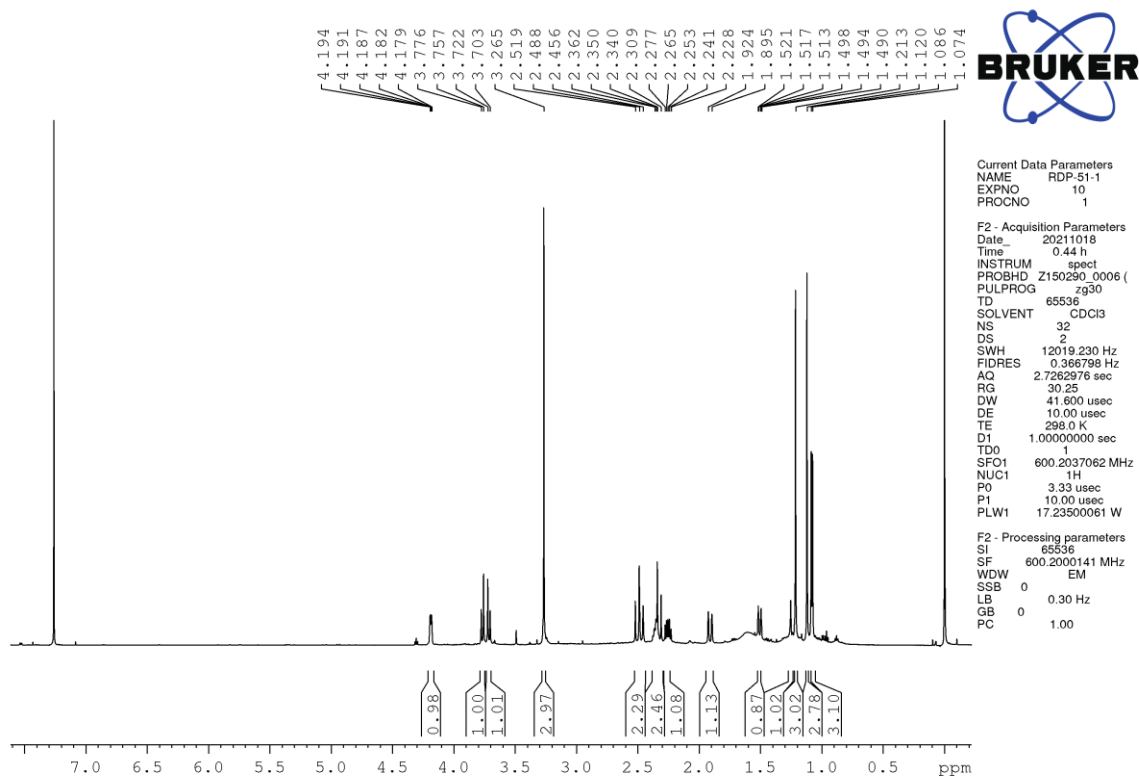


Figure S59 ^1H NMR spectrum (600 MHz, CDCl_3) of compound **8**

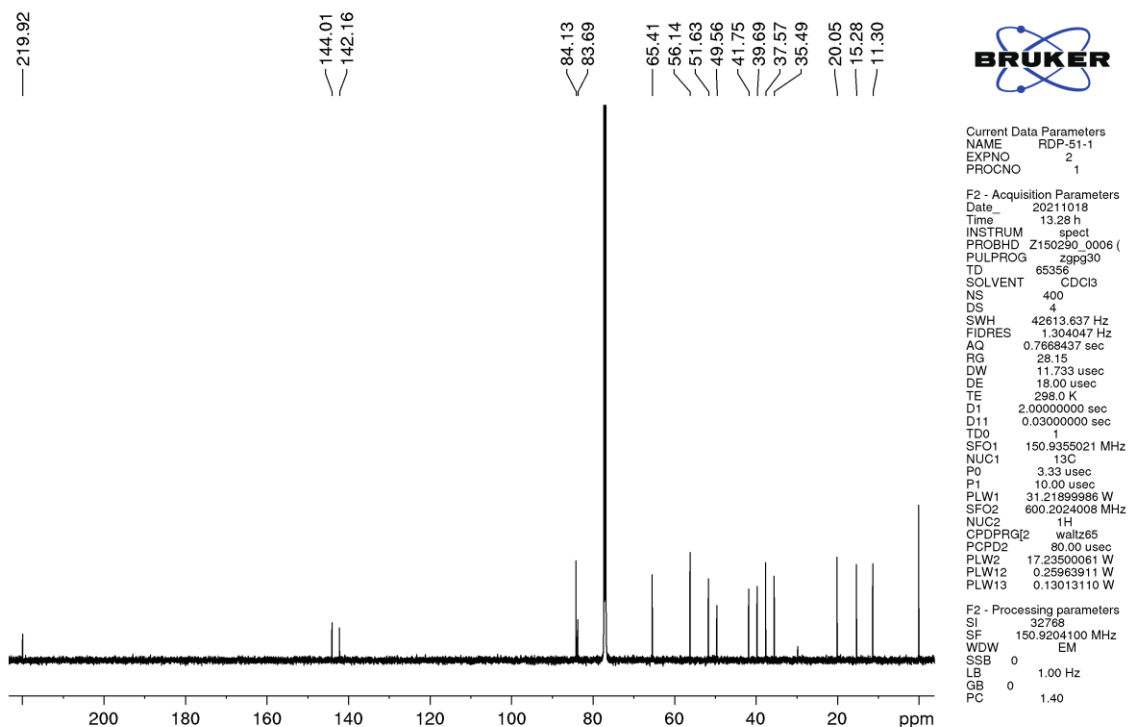


Figure S60 ^{13}C NMR spectrum (150 MHz, CDCl_3) of compound **8**

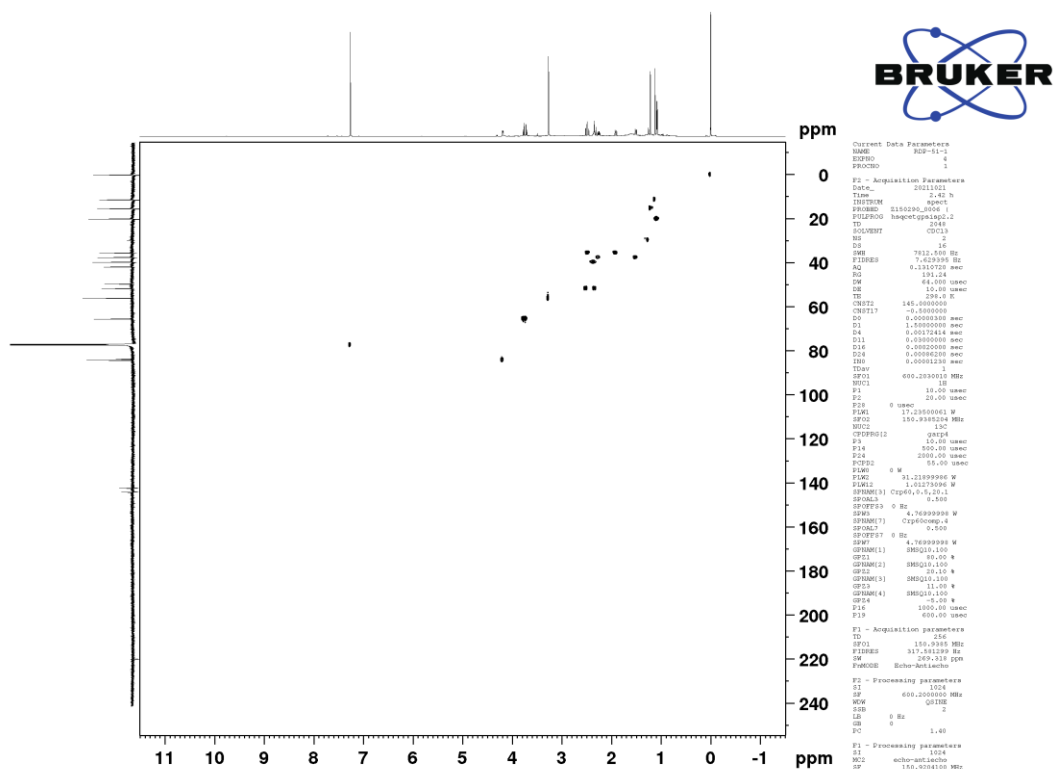


Figure S61 HSQC spectrum (600 MHz, CDCl₃) of compound 8

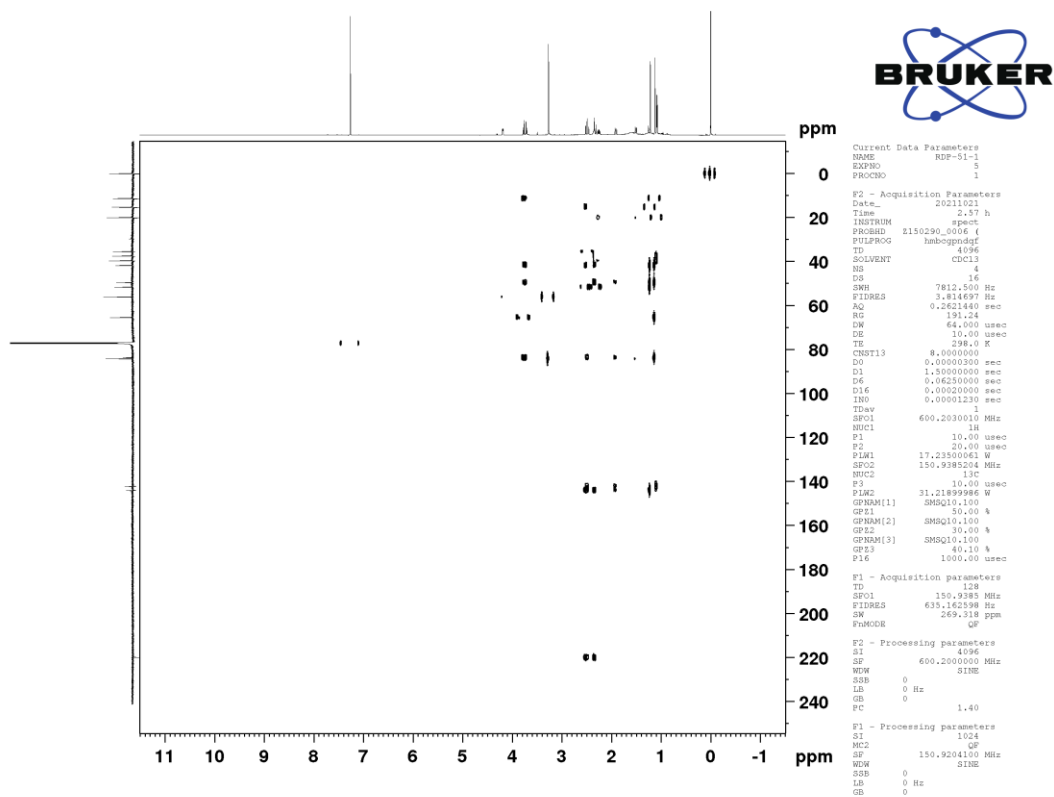


Figure S62 HMBC spectrum (600 MHz, CDCl₃) of compound 8

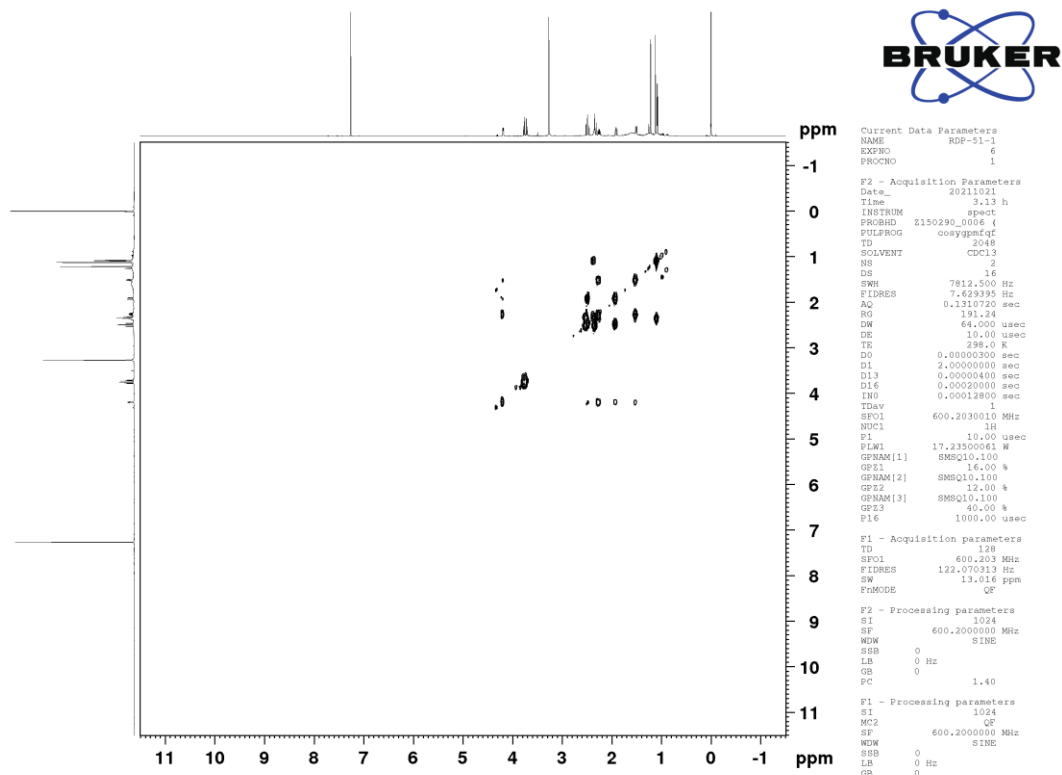


Figure S63 ^1H - ^1H COSY spectrum (600 MHz, CDCl_3) of compound **8**

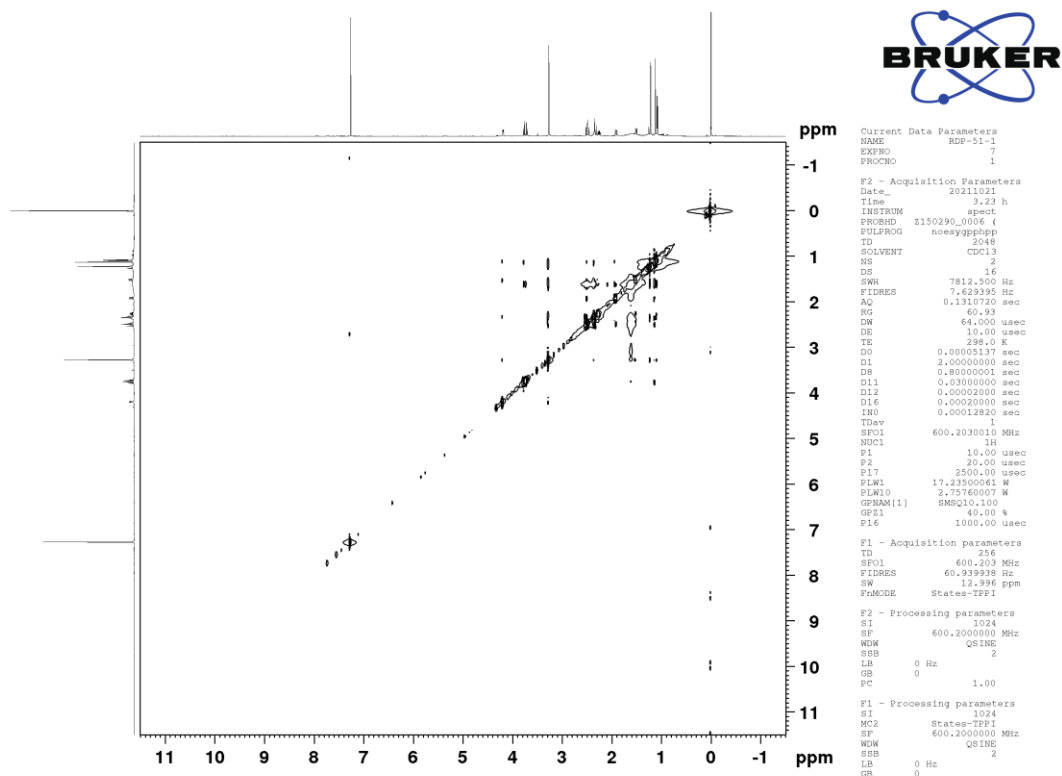


Figure S64 NOESY spectrum (600 MHz, CDCl_3) of compound **8**

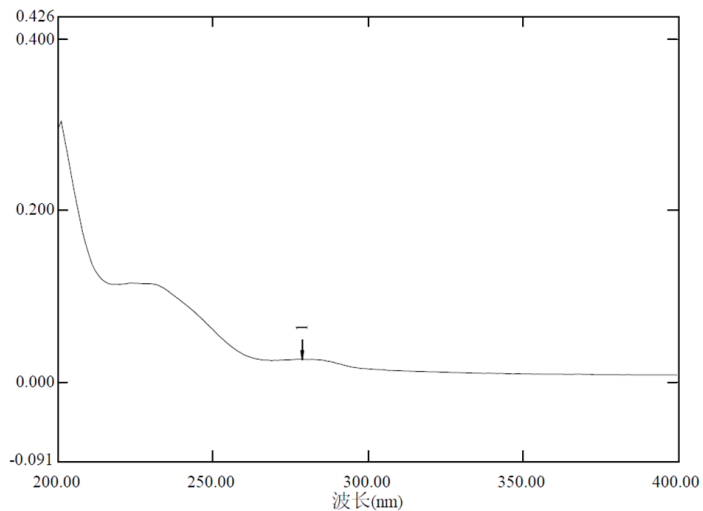
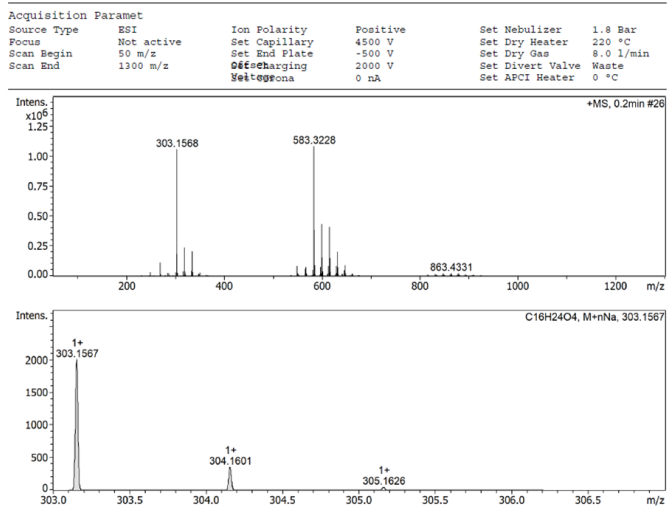


Figure S65 HRESIMS and UV spectra of compound 9

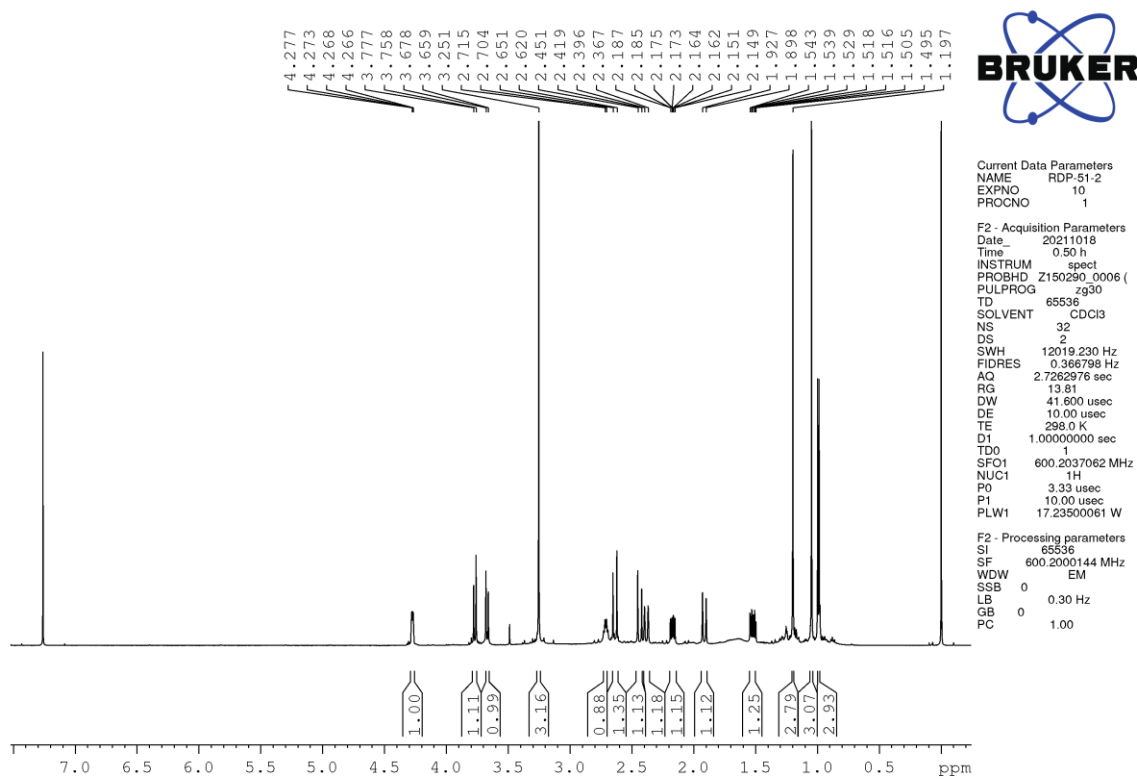


Figure S66 ^1H NMR spectrum (600 MHz, CDCl_3) of compound 9

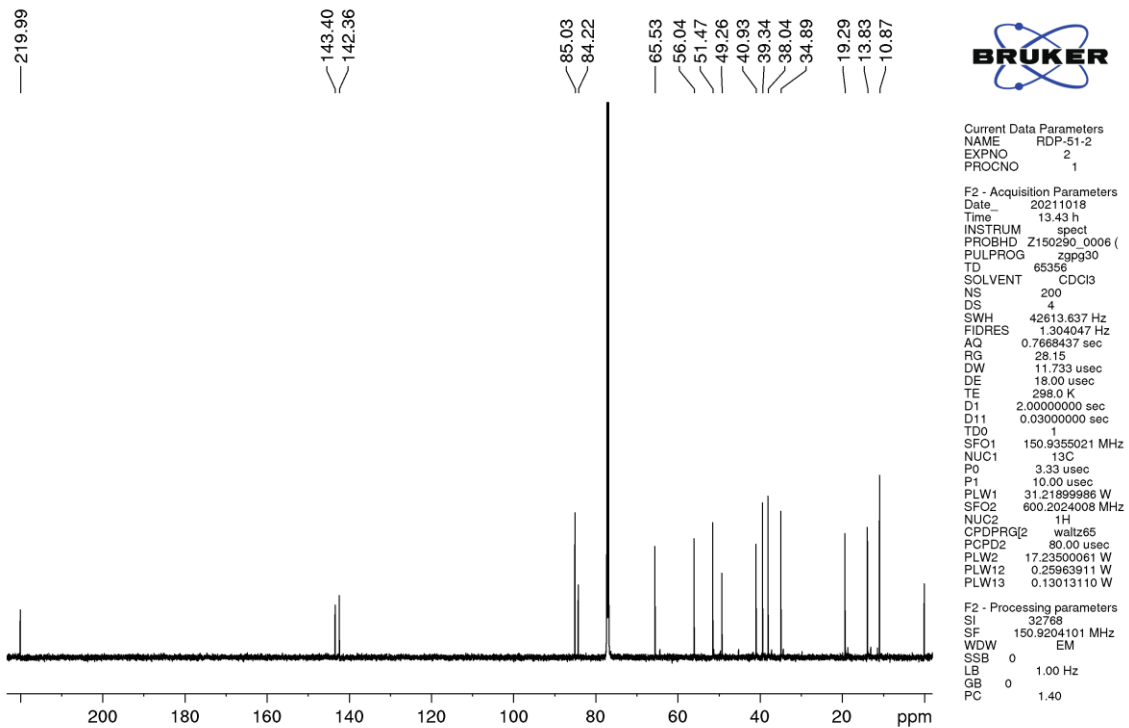


Figure S67 ^{13}C NMR spectrum (150 MHz, CDCl_3) of compound 9

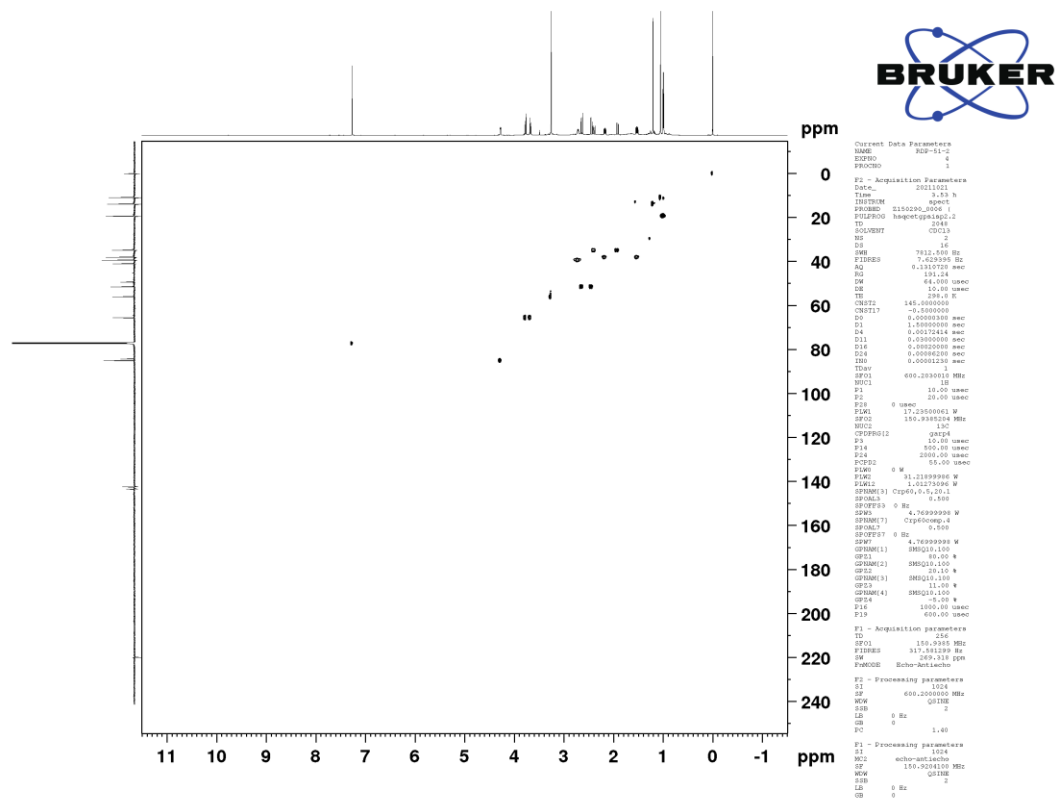


Figure S68 HSQC spectrum (600 MHz, CDCl_3) of compound 9

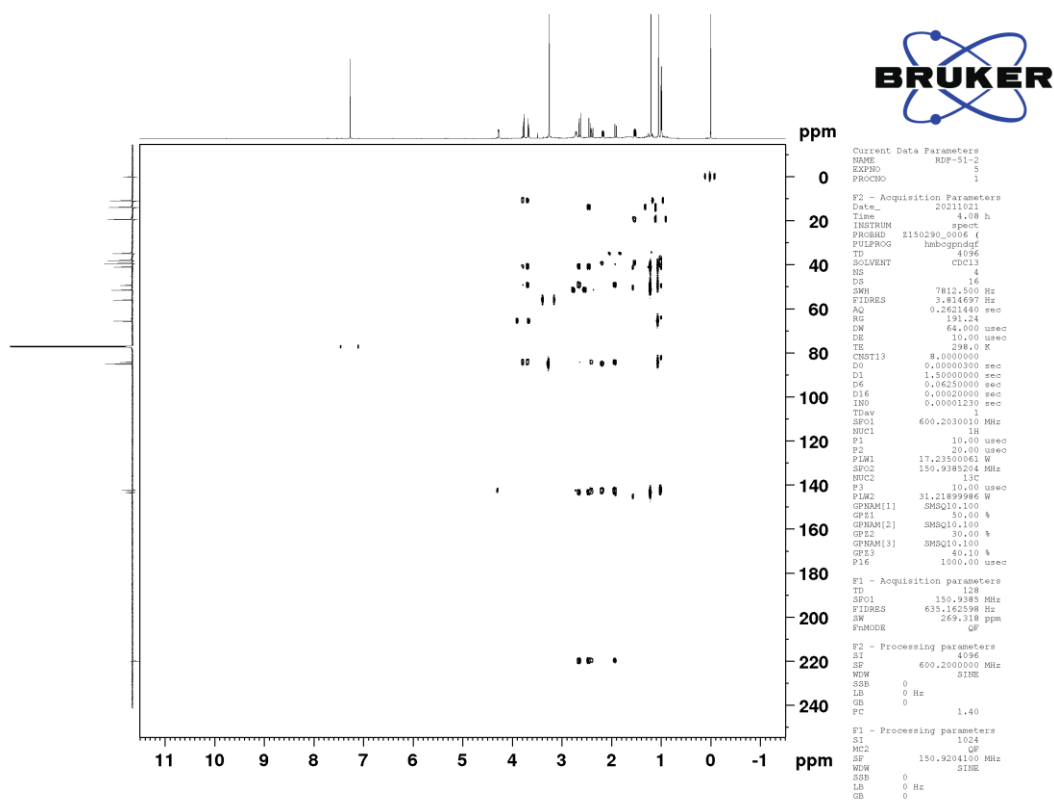


Figure S69 HMBC spectrum (600 MHz, CDCl₃) of compound 9

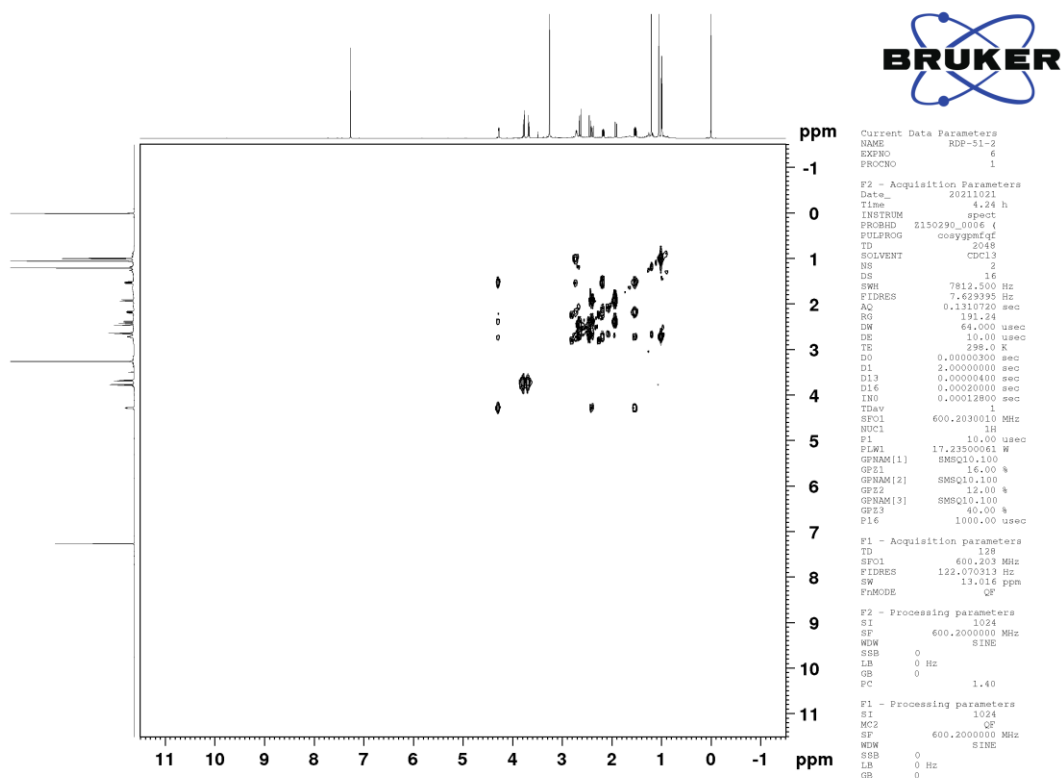


Figure S70 ¹H-¹H COSY spectrum (600 MHz, CDCl₃) of compound 9

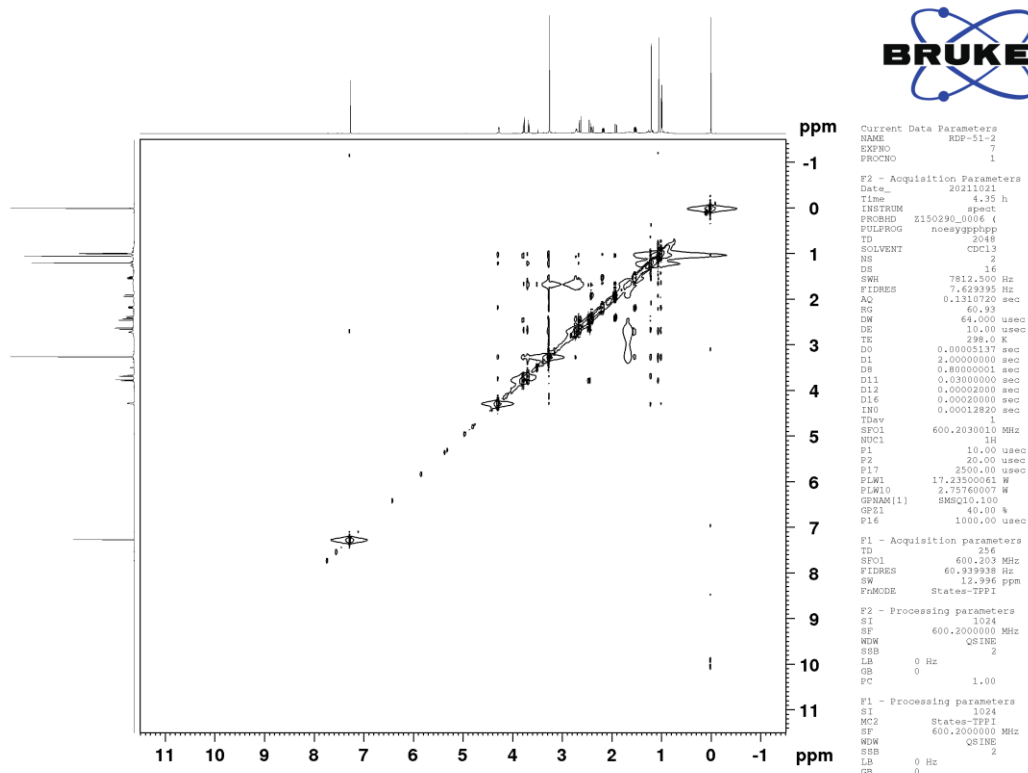


Figure S71 NOESY spectrum (600 MHz, CDCl₃) of compound **9**

Table S1 Cytotoxicities of compounds **1–11** against Hep3B and HepG2 cells (IC₅₀, μM)

compound	Hep3B	HepG2	compound	Hep3B	HepG2
1	>50	>50	7	37.45 ± 0.42	>50
2	21.66 ± 1.82	>50	8	17.11 ± 0.78	>50
3	33.90 ± 0.92	>50	9	17.95 ± 1.12	>50
4	22.11 ± 1.98	>50	10	>50	>50
5	37.53 ± 0.88	>50	11	33.42 ± 1.38	>50
6	36.36 ± 0.29	>50	sorafenib ^a	5.48 ± 0.25	11.67 ± 0.58

Data presented as Mean ± SD (n = 3).

^a Positive control